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**PROCEEDINGS OF THE
1994 SUGAR PROCESSING
RESEARCH CONFERENCE**

**AUGUST 7-9, 1994
HELSINKI, FINLAND**

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1994 SUGAR PROCESSING
RESEARCH CONFERENCE**

**AUGUST 7-9, 1994
HELSINKI, FINLAND**

**Sponsored by
Sugar Processing Research Institute, Inc.**

December, 1994

THE SUGAR PROCESSING RESEARCH
INSTITUTE, INC.
1000 N. 10TH AVE., SUITE 100
DENVER, CO 80202

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INSTITUTE, INC.

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PREFACE

The 1994 Sugar Processing Research Conference is one of a series of Conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments. The Conference was sponsored by Sugar Processing Research Institute, Inc. (SPRI). The Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, contributed in kind to the organization of the Conference.

The program for this conference was arranged by Margaret A. Clarke. The Conference Coordinator was Shirley T. Saucier. These Proceedings were edited by Margaret A. Clarke with editorial assistant Beryl Ann Borel.

The series, Proceedings of the Sugar Processing Research Conference, of which this is the seventh issue, continues the Proceedings of the Technical Sessions on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume as well as back issues of the former series as long as the supply lasts, write to Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, LA 70124. Before 1986, Proceedings were published by the Agricultural Research Service, U.S. Department of Agriculture. Since 1988, Proceedings have been published by the Sugar Processing Research Institute.

Margaret A. Clarke
Managing Director
Sugar Processing Research Institute, Inc.

Leif Ramm-Schmidt
President
Sugar Processing Research Institute, Inc.

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1994 S.P.R.I. SCIENCE AWARD PRESENTATION

COMPUTER SIMULATION OF CHEMICAL AND BIOLOGICAL PROPERTIES OF SUCROSE, THE CYCLODEXTRINS AND AMYLOSE

Frieder W. Lichtenthaler

Institute of Organic Chemistry, Technical University of Darmstadt, D-64287 Darmstadt, Germany

ABSTRACT

Computer-aided modeling of the conformations, the molecular electrostatic potential (MEP), and the molecular lipophilicity potential (MLP) profiles are presented for sucrose, fructose, non-carbohydrate sweeteners, cyclodextrins, and the amylose portion of starch. The visualization of their MEP and MLP patterns on the solvent-accessible surface in color-coded form provides novel insights into the molecular architecture of these molecules, their chemical properties (e.g. acidity of OH groups), and receptor interactions in eliciting biological responses, such as sweetness.

INTRODUCTION

The availability of advanced computer modelling techniques, their application to the elucidation of the individual conformations of carbohydrates in vacuum and in solution (15), particularly the possibility of representing various properties on the contact surface of sugars (31,32) have added a new dimension to the visual perception of sugars. Accordingly, not only may the electropositive and electronegative areas on the surface of a sugar molecule be reliably determined by computational methods, but the hydrophilic and hydrophobic regions as well (31,32).

Such information is particularly useful for understanding and, hence, predicting hydroxyl group reactivities in sugars, inasmuch as they are similar or nearly identical in the multitude of cases. Here, an insight into the relative acidities of the individual hydroxyl groups, which may be gained from the pattern of the molecular electrostatic potential (MEP) on the contact surface of a sugar, is apt to induce straightforward strategies for planning selective entry reactions into useful derivatives.

In a similar fashion, reliable knowledge on where a mono- or disaccharide is hydrophobic - in principle obtainable from computation of their MLP's (molecular lipophilicity potentials) - has major implications on its biological properties, inasmuch as the "docking" of a substrate in a receptor (e.g. sucrose in the taste bud) is governed by hydrophobic interactions between the respective part of the substrate and the complementary hydrophobic section of the receptor protein. Only when this docking is

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complete, the biological response (i.e. sweetness in the case of sucrose) is elicited, most probably by hydrogen bonding via another part of the substrate.

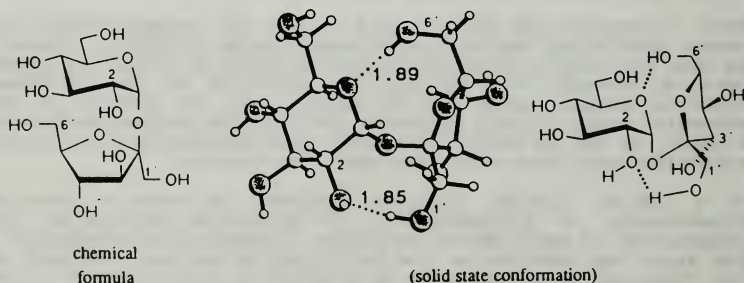
To secure the MEP's and MLP's of simple sugars and disaccharides is thus a most helpful tool with which to plan chemical reactions of high potential selectivity, and to understand their biological processes on a molecular level with the ultimate aim to modify intentionally their biological response (e.g. intensifying sweetness).

This account gives an overview on the present state of computer-aided modelling and visualization of the conformations, the solvent accessible surfaces and the MEP's and MLP's of simple sugars such as sucrose - non-carbohydrate sweeteners included for comparison - and extends these investigations to the cyclodextrins and the amylose portion of starch in order to get a better understanding of their molecular architecture and their properties.

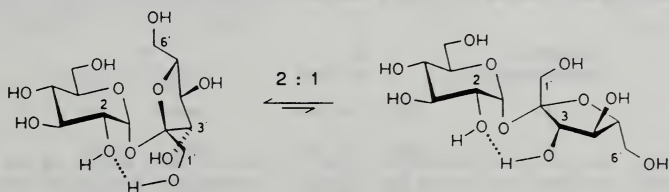
RESULTS AND DISCUSSION

Conformation of Sucrose in the Solid State and in Solution

In Figure 1, the common sucrose formula (left) does not give any three-dimensional information about the actual predominating conformation (32). A more "realistic" molecular picture is obtained from neutron diffraction (6), and from X-ray analysis (19) of the solid-state conformation (right), showing the glucose and fructose moieties fixed in their relative orientation by two strong interresidue hydrogen bonds between 5-O⁴...HO-6' and 2-O⁴...HO-1' of 1.89 and 1.85 Å length, respectively:



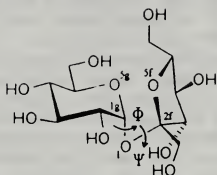
In solution, particularly in water, it is unlikely that both intramolecular hydrogen bonds of the crystalline state are retained; indeed, extensive ^1H - and ^{13}C -NMR investigations (4,10, 11,40,41) strongly attest to the disintegration of the $5\text{-O}^2\cdots\text{HO-6}'$ hydrogen bond by solvation, despite the fact that a more recent NMR-study arrives at the peculiar conclusion that there is no intramolecular hydrogen bond in sucrose in aqueous solution (1). Detailed optical rotation data of aqueous sucrose solutions (51), however, are best accounted for in terms of an equilibrium mixture of two conformers, the predominant one being similar to the crystalline structure in which the $2\text{-O}^2\cdots\text{HO-1}'$ hydrogen bond persists (left formula), and the other featuring a $2\text{-O}^2\cdots\text{HO-3}'$ intramolecular hydrogen bond (right):



Sucrose: conformation in solution

This contention is in accord with conclusions, reached on the basis of steady state NOE's and long-range ^{13}C - ^1H couplings, that sucrose in aqueous solution maintains the $2\text{-O}^2\cdots\text{HO-1}'$ intersaccharidic hydrogen bond (4), as well as with SIMPLE NMR-based reasonings, that, in DMSO solution, a $2 : 1$ equilibrium of the two conformers prevails (10).

A variety of computational methods and force fields have been used for the theoretical treatment of sucrose towards unravelling its minimum energy conformations, such as HSEA (4), PFOS (13), CHARMM (52,53), PIMM88 (35) and MM3 (16). Despite the fact that the locations and relative energies of the minima differ to some extent, some trends are independently reproduced by all studies: of the intersaccharidic torsion angles Φ and Ψ (definition see formula below), the former, representing rotation around the axially oriented anomeric $\text{C}_{1\text{A}}\text{-O}_1$ bond of glucose is considerably more restricted than the latter (Ψ), which describes the flexibility around the alternate pseudoequatorial $\text{C}_{2\text{A}}\text{-O}_1$ -anomeric bond of the fructose portion.



Sucrose: definition of intersaccharidic torsion angles

$$\Phi = O_{5g}-C_{1g}-O_1-C_{2f}$$

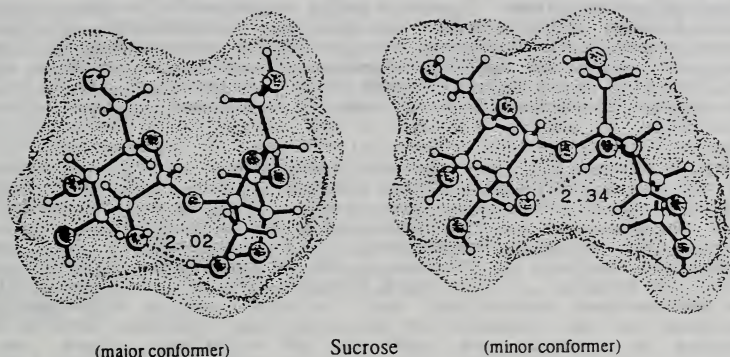
$$\Psi = C_{1g}-O_1-C_{2f}-O_{5f}$$

In all cases, three main families A - C of sucrose minimum energy conformers are found with Φ/Ψ -angle ranges

sucrose conformers	Φ	Ψ
A	+70 - +110°	-40 - -90°
B	+80 - +100°	+170 - -170°
C	+90 - +120°	+50 - +70°

whereby the global energy minimum conformer A closely resembles the solid state conformation, and the minima B and C are higher in energy by 8 - 25 kJ/mol.

These rationalizations, from the bulk of calculatory evidence accumulated to date, correspond to our results obtained with the PIMM88 force field program (37), which appears to be particularly well suited for the treatment of overlapping anomeric effects. Computation of the fully relaxed energy potential surface provided three local energy minima for sucrose (Figure 1) in a percentage distribution of 71 : 21 : 8% (35). Thereby, the major conformer is characterized by its 2-O^g...HO-1' hydrogen bond, the minor one features the 2-O^g...HO-3' alternative, whilst the third - of 8% population only - exposes a geometry determined by a hydrogen bond between the fructose 3-OH and the glucose ring oxygen. The molecular geometries of these conformers are depicted in Figure 1; the two major ones are again given below in ball and stick representation, yet with their contact surfaces superimposed in dotted form:



It is to be noted that these two major conformers emerging from our PIMM calculations correspond closely to those delineated from the NMR and rotational data mentioned above. Accordingly, we based our modellings of the electrostatic and hydrophobic properties on these two molecular geometries.

Contact Surface and Molecular Electrostatic Potential (MEP) Profile of Sucrose

The molecular geometries elaborated above for sucrose should conceivably be brought to a test, i.e. whether they are able to explain, or even predict chemical and/or biological properties. With this in mind, we have generated, by use of the MOLCAD program (5) the so-called contact surfaces of these conformers (dotted areas above) relative to water molecules, i.e. "how water sees sucrose". For each of these dots on the surface, the Molecular Electrostatic Potential ("MEP") was calculated (i.e. how positive or negative in electrostatic terms such a surface point is), and the respective numeric values were transferred into a color-code for visualization (35).

The resulting MEP patterns for each the two relevant sucrose conformers are given in Figure 2. As is clearly evident, the area of the 2-OH group of the glucose portion - the most intense red surface part - is the one with the highest positive electrostatic potential. This necessarily entails the notion that the strong positivation of the glucosyl-2-OH must lead to enhanced acidity of

this hydroxyl group over the others, i.e. on base treatment, should be deprotonated first. There is ample experimental evidence that this, indeed, is the fact. It has been demonstrated (9) that 2-O-acyl and 2-O-(N-carbamoyl)-derivatives of sucrose are obtained in useful yields by NaH-deprotonation of sucrose in pyridine and reaction with 3-acylthiazolidinethiones. Our studies (44) on this NaH-induced deprotonation of sucrose and subsequent exposure of the mono-anion to benzyl bromide in DMF opened up a large scale adaptable route towards 2-O-benzyl-sucrose: on the reaction with benzyl bromide a 9 : 2 : 1-mixture of monobenzyl ethers is generated, which are the 2-O-benzylated product (major), and the fructose-1'-O- and 3'-O-benzylisomers (minor). Similar regioselectivities are observed on electroreductive deprotonation of sucrose within the cathodic compartment of an electrolysis cell and subsequent trapping of the mono-anion by alkylation or acylation (18). In essence, all of these findings are well in accord with the MEP-derived predictions, and, in turn, provide experimental evidence for the retention of intramolecular hydrogen bonding in sucrose in solution (at least in DMF solution), since, very obviously, the interresidue 2-O⁻...HO-1' and 2-O⁻...HO-3' hydrogen bonds in sucrose not only determine the MEP profiles, i.e. the electro-positive 2-OH proton, but are also responsible for the stabilization of the anion at these positions (35).

Molecular Lipophilicity Potential (MLP) Profiles of Sucrose

The interactions of sugars with receptor proteins are not only governed by electrostatic effects, including hydrogen bonding phenomena, but also by dispersion forces (van der Waals contacts) and hydrophobic effects. However, when looking at standard space-filling models, the assessment of this kind of interaction, i.e. the areas that might be hydrophobic, is difficult if not actually impossible. Utilizing the MOLCAD-program (5), the Molecular Lipophilicity Potentials (MLP's) can be calculated on molecular surfaces, i.e. the hydrophobicity portraits of the respective molecules.

Applying this methodology to sucrose (32,33), the MLP profiles for both relevant conformers were generated and the computed values were transferred into a two color-code graded into 32 shades (Figure 3). The color-coding was adopted to the range of relative hydrophobicity calculated for each molecule, using 16 colors ranging from dark blue (most hydrophilic surface areas) over light blue to full yellow (most hydrophobic regions) for mapping the computed values on the surface. The remaining 16 color shades (light blue to brown) were used to indicate iso-contour lines in between former color scale, allowing for a more quantitative assessment of relative hydrophobicity on different surface regions.

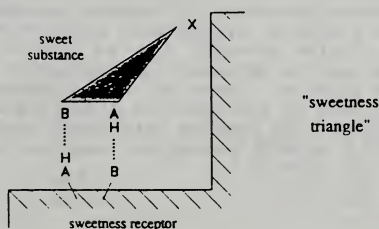
From the color representation of the MLP's for the major sucrose conformation in Figure 3 (left entry), the hydrophilic and hydrophobic portions of the molecule are distinctly separated on opposite sides. Particularly clear is the half-opened form with the stick and ball-model insert, revealing the entire outside section of the fructose moiety to be hydrophobic (i.e. yellow), and the blue hydrophilic section to be centered around the 3-oxygen of glucose.

Inspection of the alternative sucrose conformation likely to prevail in solution (cf. Figure 3 right), reveals few changes in the overall MLP-pattern, except that the hydrophobic region located at the outer side of the fructose moiety is now more compact. By consequence, that part of sucrose able to engage in hydrophobic binding within the sweet-taste receptor is to be assigned to an entire region of the fructose portion, rather than a specific position (cf. below).

Biological Significance of MLP Profiles of Sucrose

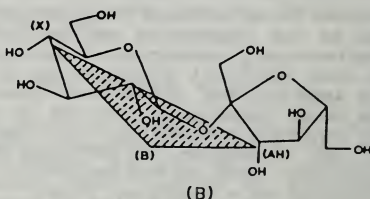
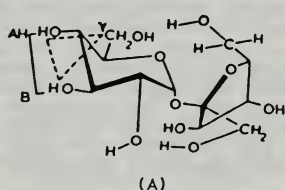
Sucrose being actively transported in plants, its recognition binding to the respective carrier proteins is governed not only by complementary geometries of substrate and receptor, but by similar or even identical topographies in hydrophilic/hydrophobic terms. The same applies, maybe even more rigidly, to the elicitation of the sweet response by sucrose in the taste bud. The knowledge of the relevant hydrophilic and hydrophobic regions on the contact surface of sucrose, as provided by the MLP patterns of Figure 3, sheds new light on the notions of how the sweetness sensation is triggered on a molecular level.

The classical attempt by Shallenberger (49) and Kier (28) to rationalize the sweet taste of organic compounds presumes the existence of a common AH-B-X glucophore in all sweet substances, eliciting the sweet response via the interaction with a complementary tripartite AH-B-X site in the taste bud receptor (30):



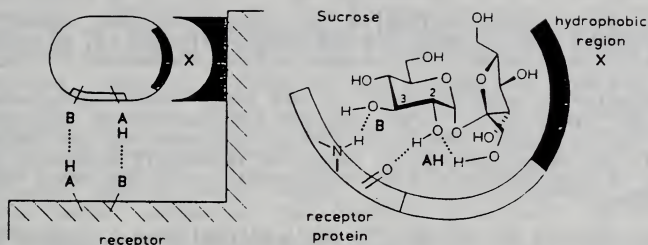
This theory, however, also termed the 'sweetness triangle', appears much too simple to explain all of the observations, particularly when bearing in mind, that sweet-taste perception is mediated by a cascade of complex biochemical processes (12,14,29,50) that are little understood at the cellular level. Nevertheless, the tripartite AH-B-X glucophore concept has had its merits as a unifying criterion and proved useful - despite its neglect of three-dimensional shape and volume - in rationalizing structure--sweetness relationships in such diverse classes of compounds as amino acids, dipeptides, sulfamides (e.g. saccharin and acesulfame), and sugars in particular, most notably the natural sweeteners sucrose and fructose.

In the case of sucrose, for example, the assignment of the AH-B-X site for sweetness elicitation has been encumbered by uncertainties in placing the "sweetness triangle", i.e. in correlating the respective AH-, B-, and X-parts to distinct parts of the molecule, as assignments A (38,39) and B (22) -depictions from the original literature, respectively - have been proposed:



However, neither of these concepts have been able to explain the sweetness characteristics of a large number of sweet sucrose derivatives, nor do they have any predictive value (30).

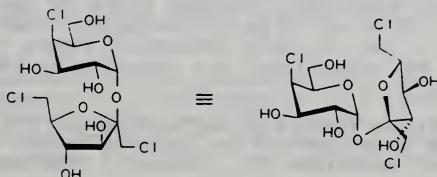
The MLP patterns presented in Figure 3 provide ample evidence, that there is not a single, hydrophobic binding point as implied by the sweetness triangle concept, but an entire extended hydrophobic region in the sucrose molecule, encompassing the entire backside of the fructose portion. Moreover, the hydrogen bonding AH-B-couple must be contained in the opposite side-located, hydrophilic region of the molecule. In keeping with these notions, the location of the tripartite AH-B-X glucophore emerges in the following form (33):



Accordingly, the AH-B-couple is represented by the glucosyl-2- and 3-OH groups, of which the distinctly electropositive glucosyl-2-OH (cf. MEP profile in Figure 2) is energetically most favored to engage in hydrogen bonding as a donor, and correspondingly, may be assigned as the AH-unit. The hydrogen bond acceptor (B-unit) must be located in its direct vicinity, pointing towards the glucosyl-3-OH as the most likely candidate; the X part is represented by the entire backside of the fructose residue that contributes to the hydrophobic interaction with the sweetness receptor.

MLP Profiles of Sucralose and some Non-carbohydrate Sweeteners

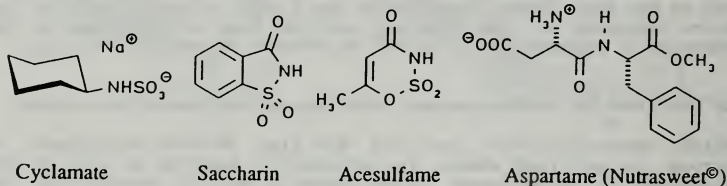
Whilst absolute proof for the modified AH-B-X assignment to sucrose, as outlined above, is necessarily lacking as long as the topography of the receptor is not known in detail, support for its validity is derived from the sweetness characteristics of altogether 53 sucrose derivatives which correlate well with this concept in terms of structure-sweetness relationships (33) - structure being used here not only in terms of geometrical implications, but also in the hydrophilic/hydrophobic profile on the contact surface. Most notably, the intensely sweet (650 x as compared to sucrose)



Sucralose

sucralose, a trichloro-derivative of galacto-sucrose, shows a hydrophobicity distribution in its MLP profile (Figure 4), in which the two chlorine atoms of the fructose portion - expectedly - turn out to be the most hydrophobic (yellow) regions (X-area). Another obvious similarity with sucrose is the fact, that hydrophobic (yellow) and hydrophilic (blue) regions are located on opposite sides of the molecule, seemingly little disturbed by the third chlorine in the galactose portion which, as is clearly evident from Figure 4, is substantially less hydrophobic than the other two halogen atoms.

For further probing the validity of our modified AH-B-X concept as a relevant working tool in structure-sweetness considerations, it was obvious to extend the MLP pattern approach to other sugars, e.g. to fructose - with favorable results (34) - and to non-carbohydrate, high-potency sweeteners, such as cyclamate, saccharin, acesulfame, and aspartame:



In the case of the three sulfamides, the similarity of their respective hydrophobicity (MLP) patterns given in Figure 5 are amazing. That the sulfamido grouping is the hydrophilic portion of the molecule was to be expected. That the differences between a cyclohexyl ring (in cyclamate), an aromatic moiety (as in saccharin) and an acetoacetyl residue fixed in the enol form (as in acesulfame) level off to yield hydrophobic areas closely resembling each other - the two lower entries in Figure 5, corresponding to saccharin and acesulfame, are essentially identical - is most remarkable.

Another striking feature is that hydrophobic and hydrophilic portions of the molecules are on opposite sites, as in the case of sucrose and sucralose (cf. above). Moreover, the very same

the aromatic ring of the phenylalanine part, whilst the amino-carboxylic acid part at the alternate side of the molecule is seemingly responsible for the hydrophilic area. The protrusion made up by the methoxycarbonyl (COOCH_3) group, conceivably not involved in binding, is apparently readily adapted by the receptor.

All of this sustains the notion that the sweet receptor - be it the same for sucrose, fructose, and non-carbohydrate sweeteners or different ones - is quite flexible in adapting to the hydrophobic portion of sweet substances, i.e. to the X part (of the tripartite AH-B-X glucophore), which clearly is not a specific position of the molecule, but an entire region. If this hydrophobic area is the main factor governing the 'docking procedure' of the sweet substance, i.e. directing it to and locking it into the complementary 'hydrophobic cleft' of the receptor protein, it can well be imagined that, thereby, the hydrophilic area of the molecule, situated on its opposite site, and likely to contain the AH-B portion of the Shallenberger-Kier tripartite AH-B-X glucophore, is brought into the appropriate position to elicit the sweet response via hydrogen bonding to a complementary receptor site AH-B couple.

In summary, much remains to be learned about the intricacies of the mechanism(s) involved in activation of sweet-sensitive cells, and direct solid evidence is urgently required. Nevertheless, the incorporation of the three-dimensional shape of sweet molecules, of their contact surfaces, and, particularly, inclusion of their MEP and MLP profiles into structure-sweetness considerations, has provided this field with a new dynamic vision, not only of the sweet molecule as such, but also of its complementary binding site. This revelation may lead, via computer-aided receptor modelling, to more realistic structure-sweetness concepts than those heretofore developed.

Hydrophobicity Potential Profiles of Cyclodextrins

The starch-derived cyclodextrins (CD's) are a group of cyclic oligosaccharides containing six, seven, eight or nine $\alpha(1\rightarrow4)$ -linked D-glucopyranose units per molecule. They have unusual bucketshaped loop-structures - a feature that allows them to form inclusion complexes by insertion of a wide variety of organic molecules into their hydrophobic intramolecular cavity (46,55) as shown in Figure 7.

Their molecular geometries were generated starting from the respective X-ray structures of the hydrates (8,17,20,56), which were allowed to "float" with the PIMM-program to obtain the following minimum energy conformation, onto which, in the methodology described for sucrose (24,25,36), the contact surfaces were

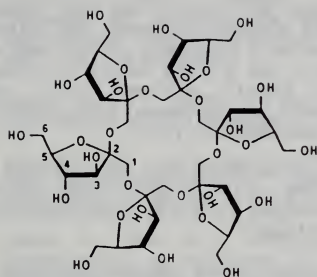
superimposed in dotted form (above formulas). The successive enlargement of the central cavity when increasing the number of glucose units from six in the cyclic hexasaccharide (α -cyclodextrin, top left) to seven (top right), eight and nine (lower entries) is as obvious, as the fact that the largest one, the δ -cyclodextrin, features a spectacle-shaped cavity rather than a uniformly circular one (25).

That these cyclodextrin cavities exhibit hydrophobic properties is well established on the basis of their ability to form 1 : 1 inclusion complexes, yet a clear indication of which areas of the macrocycle are hydrophobic versus those being hydrophilic - the cyclodextrins are fairly well soluble in water - can neither be derived from chemical formulae, nor from models or contact surfaces. Generation of their hydrophobicity potential profiles in the same way as done for sucrose (vide supra) provides a most impressive, lucid picture on how these cyclodextrins are balanced with respect to their hydrophilic (blue) and hydrophobic (yellow) areas (cf. Figure 8): one side of these molecules, i.e. the larger opening of the bucket-shaped macrocycle, carrying the secondary glucose 2-OH and 3-OH groups, is intensively hydrophilic (top entry in Figure 8), whilst the alternate narrower side (containing all of the primary CH₂OH groups) is considerably less hydrophilic (blue), partially permeated by yellow (i.e. hydrophobic) areas. The bulk of hydrophobic regions, however, is concentrated in the inner part of the cavities, as is particularly obvious in the highly symmetric γ -cyclodextrin (Figure 8, lower entry, left).

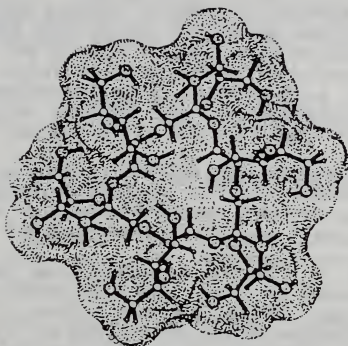
Accordingly, the complexation of the cyclodextrins with suitable guest molecules, which is governed by a variety of factors - steric fit, van der Waals, electrostatic and hydrophobic interactions - can be rationalized with respect to these factors on the basis of their MLP profiles. A detailed elaboration on the obvious implications of the MLP patterns on the ability of these cyclodextrins to form a multitude of inclusion complexes is forthcoming (25).

Cycloinulin: Geometry and Hydrophilic Topography

Enzymatic degradation of inulin, an $\beta(1\rightarrow2)$ -linked polymer of D-fructofuranose from dahlia and Jerusalem artichoke tubers, provides (analogous to the generation of the cyclodextrins from starch) cyclooligosaccharides consisting of six, seven or eight fructose residues (27,54), which are, accordingly, designated as $\beta(1\rightarrow2)$ -linked cyclofructins. The most readily accessible of these cyclofructins is the hexamer:



cyclo[D-Fruf β(1→2)]₆
("α-cyclofructin")



Based on its X-ray structure (48), the molecular geometry of the macrocycle is as depicted above (right), revealing - clearly visible on generation of the respective contact surface via the MOLCAD methodology - a topography devoid of an interior cavity (24). The color-coded visualization of the MLP profile (cf. Figure 9) indicates a distinct hydrophilic/hydrophobic differentiation between "front"- and "backside" of the macrocycle: due to the location of the fructosyl-3- and 4-OH groups as well as O-1' of the intersaccharidic linkage on the same side of the molecule (considered to be the "frontside"), this surface region is distinctly hydrophilic (blue, top entry in Figure 9); the opposite side of the macrocycle is determined by the 1-CH₂, 6-CH₂ and 5-CH fragments, and, accordingly, entails a distinctly hydrophobic "backside" (yellow areas in Figure 9, bottom entry), with an indentation in the center (24).

By consequence, the hexameric β(1→2)-cyclofructin is not capable of forming inclusion complexes with guest molecules, yet its decisively hydrophobic indentation on one side of the macrocycle is open for potential binding with complementary guests. Conceivably, the cyclofructin composed of eight β(1→2)-linked fructofuranose residues will exhibit a hydrophobic cavity large enough to allow penetration of hydrophobic guest molecules.

The Hydrophobic Topography of Amylose

Different structural models have been put forth for the amylose portion of starch: for V_H -type amylose single-stranded, left-handed helices with 6 glucose units per turn were proposed on the basis of X-ray diffraction studies (45), while the A-form seems to consist of left-handed, parallel-stranded double helices (23); both geometries and the pathway of transition between both putative conformations are still subject to discussion (47).

Using the same MOLCAD (5) program-based methodology as employed for sucrose and the cyclooligosaccharides (*vide supra*), the helical (V_H -amylose) and double-helical structures (A-form) were generated, as well as their respective MLP profiles.

As is clearly apparent from the color-coded representations in Figure 10 (top), the hydrophobic characteristics of the V_H -amylose (upper entry) and the A-form (lower entry) differ significantly, with the latter exhibiting an irregular distribution of hydrophobic and hydrophilic surface areas due to the absence of an inner channel. This is distinctly contrasted by the outside surface regions of V_H -amylose, which are highly hydrophilic (blue) while its center channel is decisively hydrophobic (yellow central portion in Figure 10, opened form in the bottom entry). The hydrophobic characteristics are in accord with the experimental finding, that amylose can form inclusion complexes with fatty acids by incorporation of their alkyl chains into the hydrophobic channel (7). Also the formation of the dark blue-stained amylose iodine-complex is caused by inclusion and an essentially perfect linear alignment of iodine/iodide in the hydrophobic center channel (Figure 10, bottommost structure), according to the solid state structure obtained from X-ray diffraction (3).

ACKNOWLEDGEMENTS

This account is the result of an intense and most delightful collaboration over the last four years with Dr. Stefan Immel who in his dissertation (24) not only met the requirements for a Ph.D. degree, but substantially surpassed them. Without his keen understanding of the calculatory programs and his superior art work visible in any of the graphics, this work would never have been accomplished. I am also grateful to Prof. Dr. J. Brickmann of this university, for kindly granting us access to his MOLCAD program (5) and the Silicon Graphics workstation.

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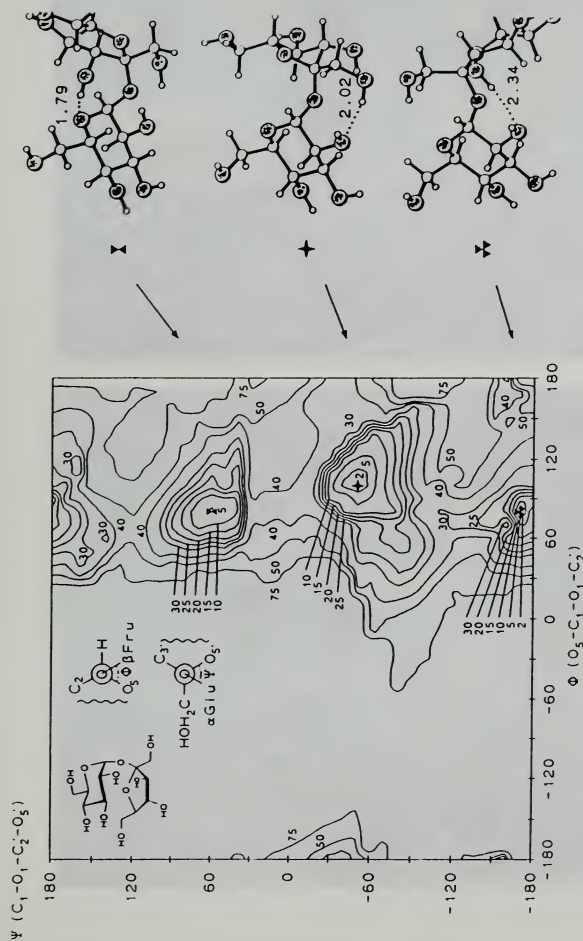


Figure 1. Fully relaxed energy potential surface of sucrose as a function of the two intersaccharidic torsion angles Φ ($O_5-C_1-O_1-C_2$) and Ψ ($C_1-O_1-C_2-O_5$), as generated by the PLUM88 force field program. Energy contours are given in kJ/mol relative to the global minimum. The energy minimum at $\Phi = +110^\circ$ / $\Psi = -50^\circ$ corresponds closely to the solid state conformation of sucrose.

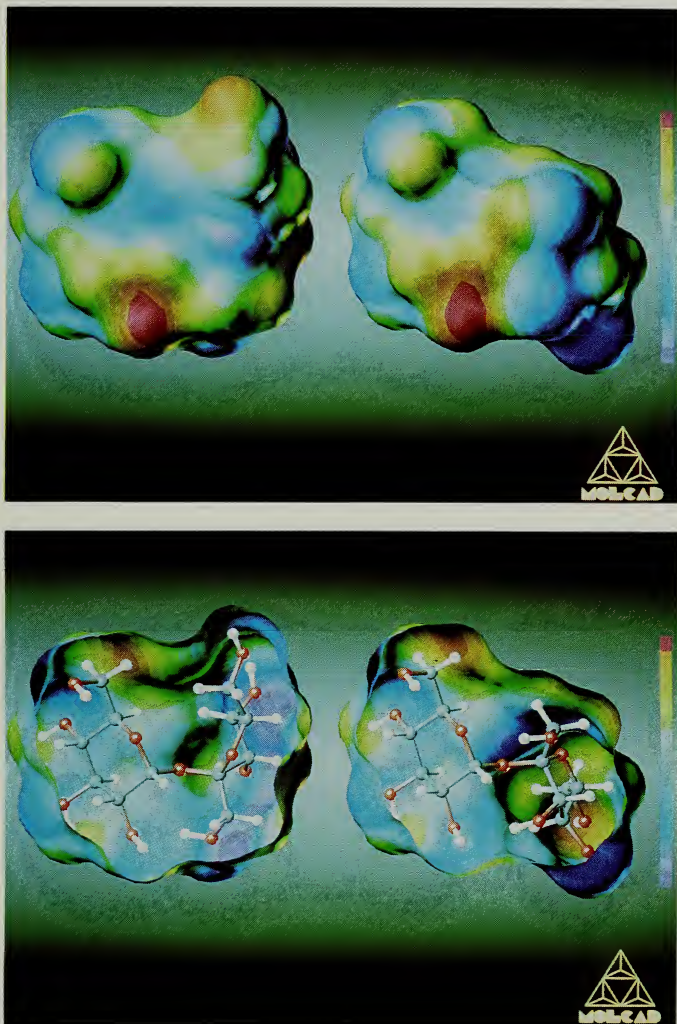


Figure 2. Representation of the molecular electrostatic potential (MEP) profiles of the two relevant sucrose conformers emerging from PIMM88 calculations (cf. Figure 1). The MEP's are depicted on the corresponding contact surfaces in a 16-color code ranging from violet (most negative potential) to red (most electropositive potential) in relative terms. To facilitate visualization, the front side-opened forms of the two conformers are also provided with a ball-stick model inserted. In either case, it is evident that the proton of the 2-OH group of the glucose part is characterized by a high positive electrostatic potential (red), indicating its enhanced acidity over other OH protons.



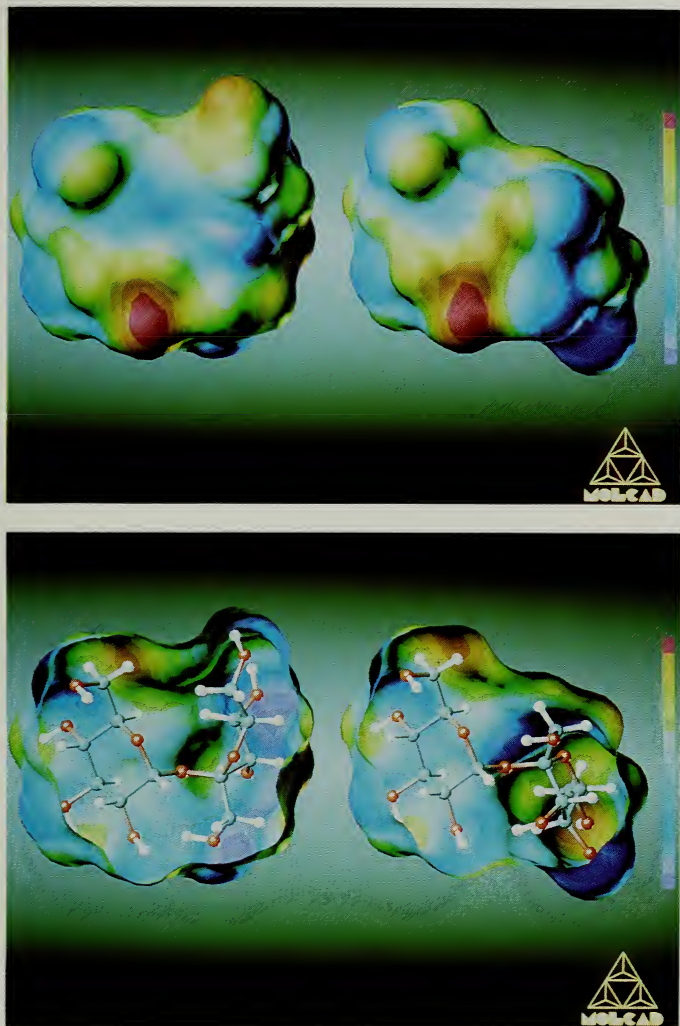


Figure 2. Representation of the molecular electrostatic potential (MEP) profiles of the two relevant sucrose conformers emerging from PIMM88 calculations (cf. Figure 1). The MEP's are depicted on the corresponding contact surfaces in a 16-color code ranging from violet (most negative potential) to red (most electropositive potential) in relative terms. To facilitate visualization, the front side-opened forms of the two conformers are also provided with a ball-stick model inserted. In either case, it is evident that the proton of the 2-OH group of the glucose part is characterized by a high positive electrostatic potential (red), indicating its enhanced acidity over other OH protons.

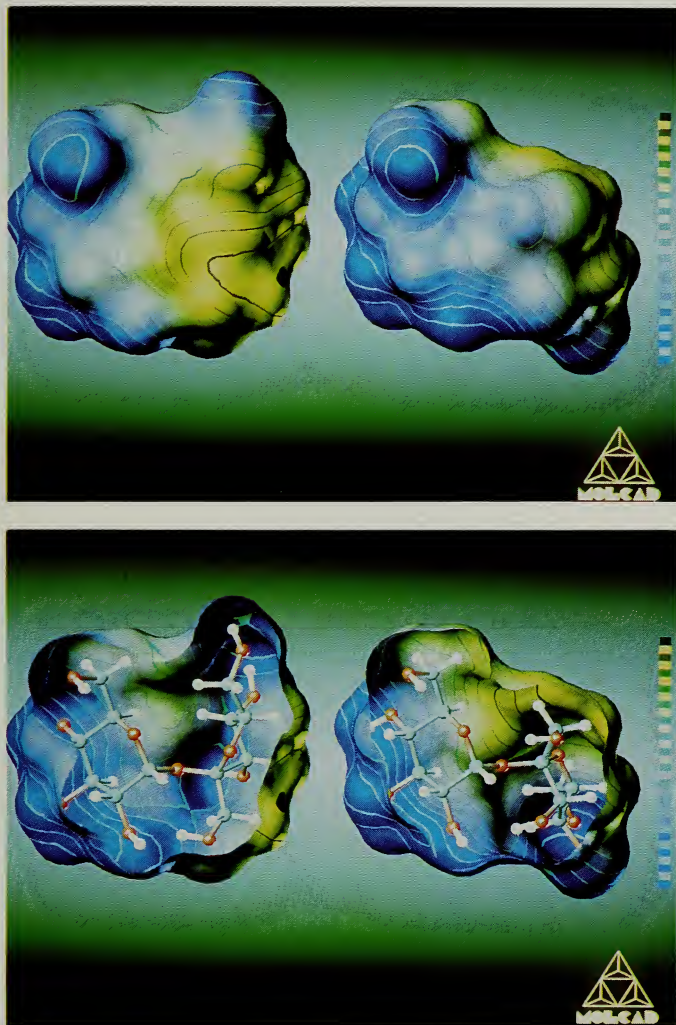


Figure 3. Molecular lipophilicity profiles for the two sucrose conformers of Figure 1, blue corresponding to hydrophilic surface areas and yellow to most hydrophobic regions. For both sucrose conformers, the entire "backside" of the fructose moiety is decisively hydrophobic.

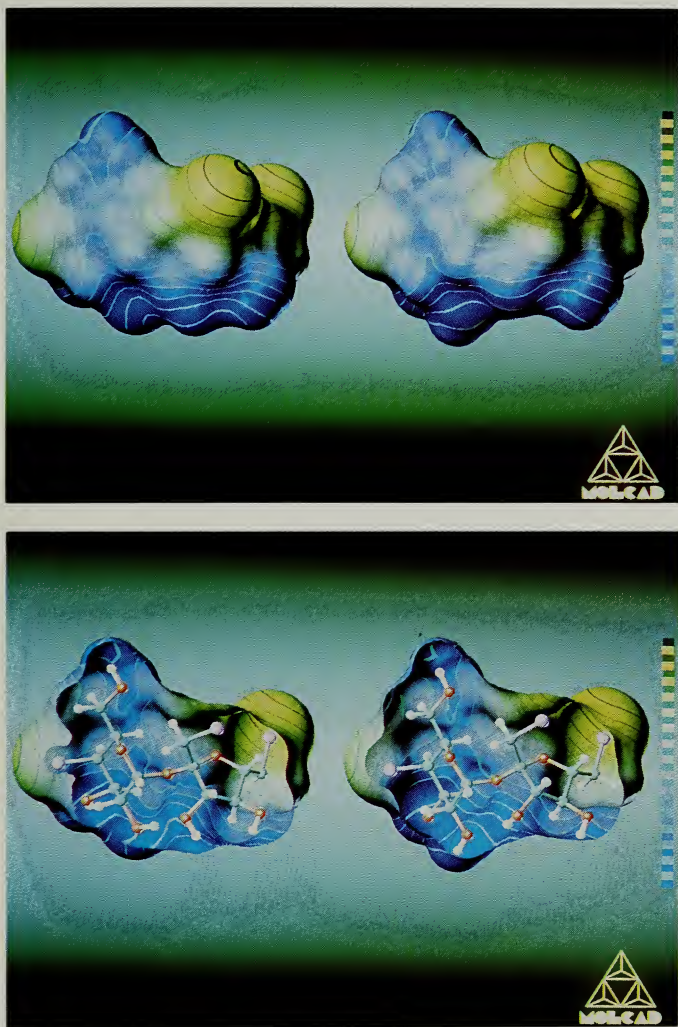


Figure 4. Molecular lipophilicity potential (MLP) patterns of sucralose (color-code cf. Figure 3) in the solid state, X-ray structure (26) derived form (left), and the computer-simulated conformation (right). The reversal of the direction of the interresidue hydrogen bond from 2-OH_g...O-3_f (left) to 2-O_g...HO-3_f (right) results in a concentration of the hydrophilic area (blue) around O-2_g.

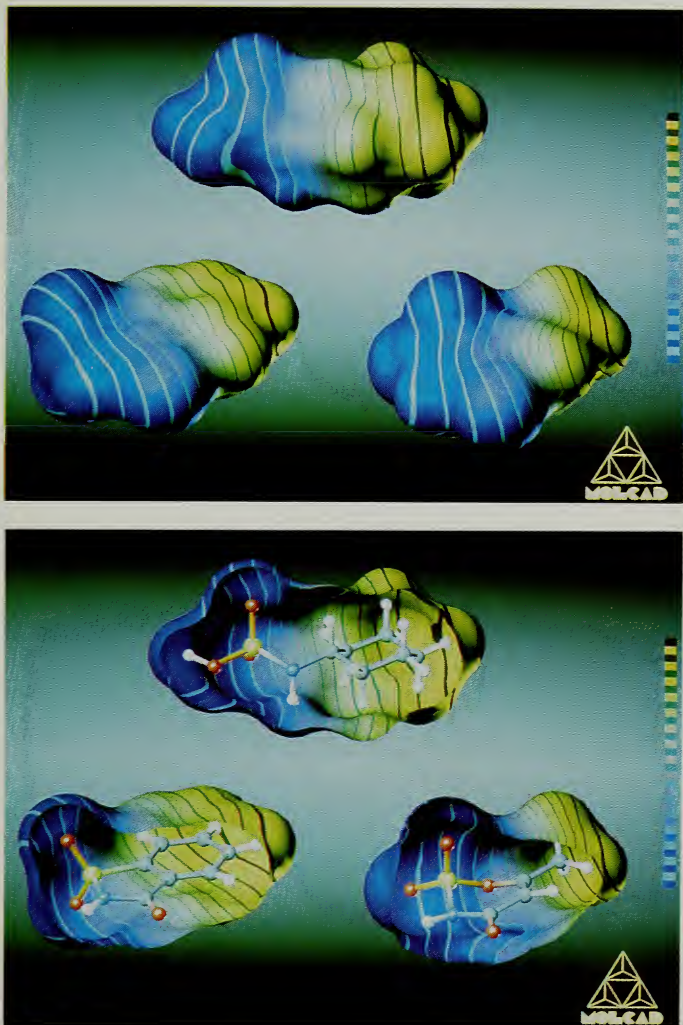


Figure 5. Color-coded representation of the molecular lipophilicity potential (MLP) profiles (yellow: hydrophobic, blue: hydrophilic regions) for the sulfamide sweeteners cyclamate (A, upper middle), saccharin (B, lower left), and acesulfame (C, lower right) in closed and opened form; the MLP's are scaled separately to the range of the hydrophobicity potential calculated onto the respective contact surfaces. The conformation of (A) was generated by force field calculations, those of (B) and (C) were modelled according to the X-ray structural data of the corresponding sodium or potassium salts (2,42,43).

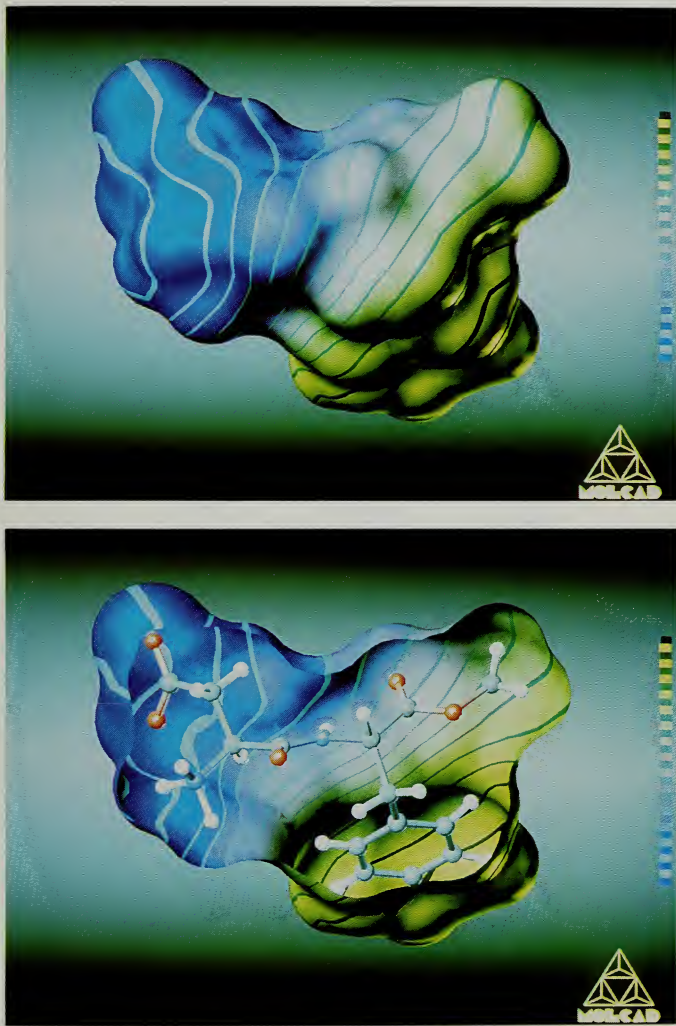
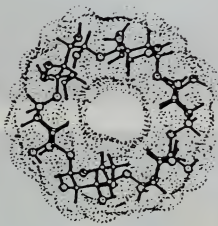
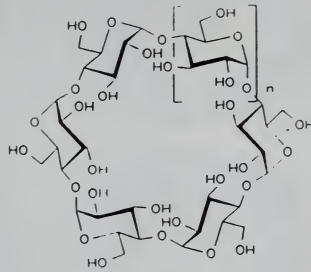


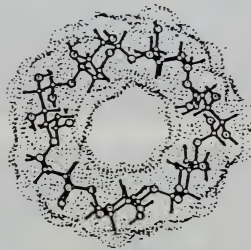
Figure 6. The MLP profile of the dipeptide sweetener aspartame in the solid state conformation (21) clearly shows the hydrophobic (yellow) region of the molecule to be determined by the benzene ring of the phenylalanine part; the hydrophilic (blue) area, seemingly originating from the amino-carboxylic acid portion of the molecule, is again in an opposite-side arrangement. The protrusion made up by the methoxycarbonyl group is obviously not involved in binding, and can apparently be readily adapted by the sweetness receptor.



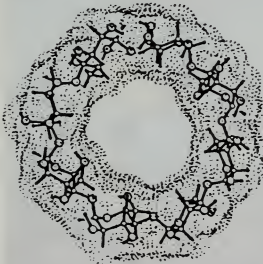
Cyclodextrins α ($n = 1$)
 β ($n = 2$)
 γ ($n = 3$)
 δ ($n = 4$)



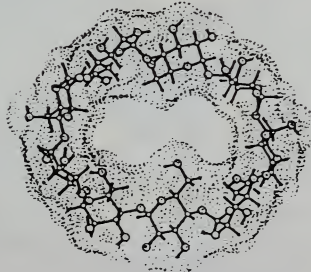
α -cyclodextrin
cyclo[D-Glcp $\alpha(1 \rightarrow 4)$]₆



β -cyclodextrin
cyclo[D-Glcp $\alpha(1 \rightarrow 4)$]₇



γ -cyclodextrin
cyclo[D-Glcp $\alpha(1 \rightarrow 4)$]₈



δ -cyclodextrin
cyclo[D-Glcp $\alpha(1 \rightarrow 4)$]₉

Figure 7. The cyclodextrins

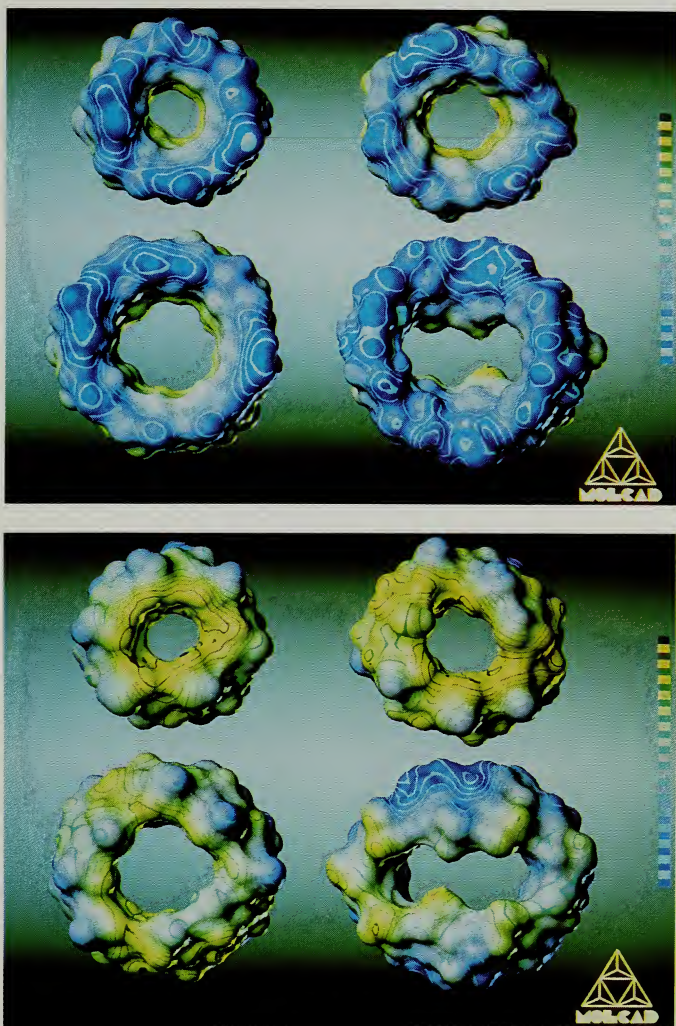


Figure 8. MOLCAD-program generated molecular lipophilicity potential (MLP) profiles projected onto the contact surfaces of α -CD (upper left), β -CD (upper right), γ -CD (lower left), and δ -CD (lower right), in their solid state conformations (8,17,20,56), respectively. The top picture views through the larger openings of the conically shaped molecules, showing the intensively hydrophilic (blue) 2-OH / 3-OH side. The bottom representation depicts the "backside" of the four cyclodextrins (i.e. the smaller opening with the CH_2OH groups facing the viewer), clearly exposing the hydrophobic (yellow) surface areas that extend well into the cavities.



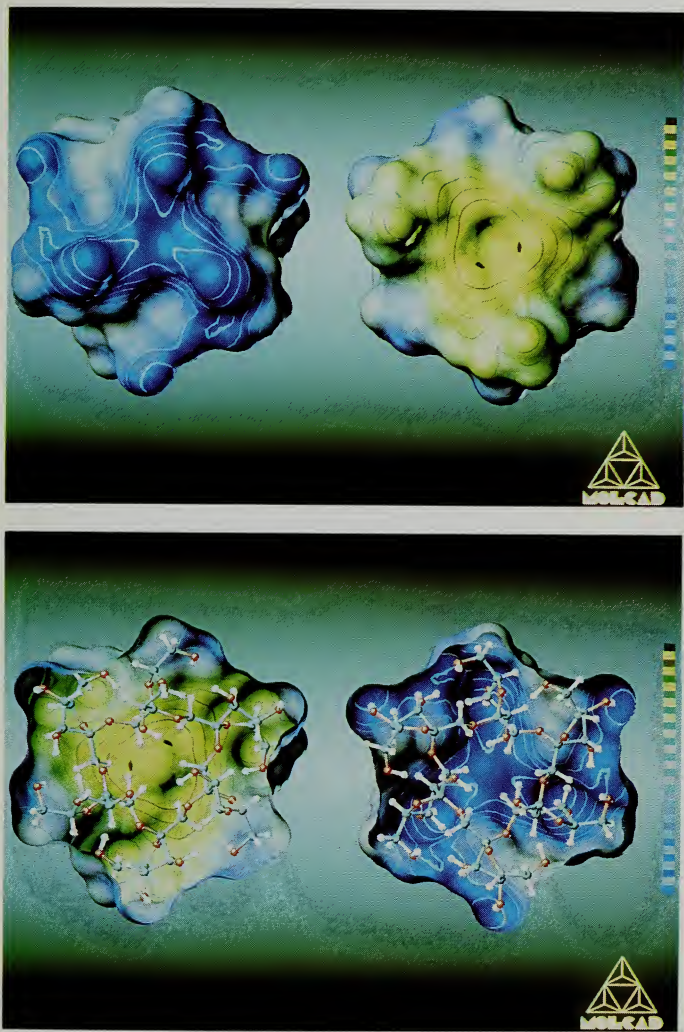


Figure 9. MLP profile (blue: hydrophilic surface regions, yellow: hydrophobic areas) of α -cyclofructin projected onto the contact surface of the solid state structure, the half-opened models with a ball and stick model inserted illustrate the molecular orientation. On the left side each, the molecules are oriented such that the 3-OHf and 4-OHf groups of the fructofuranose residues point towards the viewer, and the 6-CH₂OH groups as well as the furanose ring oxygens O-5f are directed away from him, towards the back. The right models are rotated by 180°, exposing the backsides, respectively. The separation of hydrophilic and hydrophobic surface regions on opposite molecular sides becomes clearly evident, the latter being associated with a shallow surface dent at the backside.



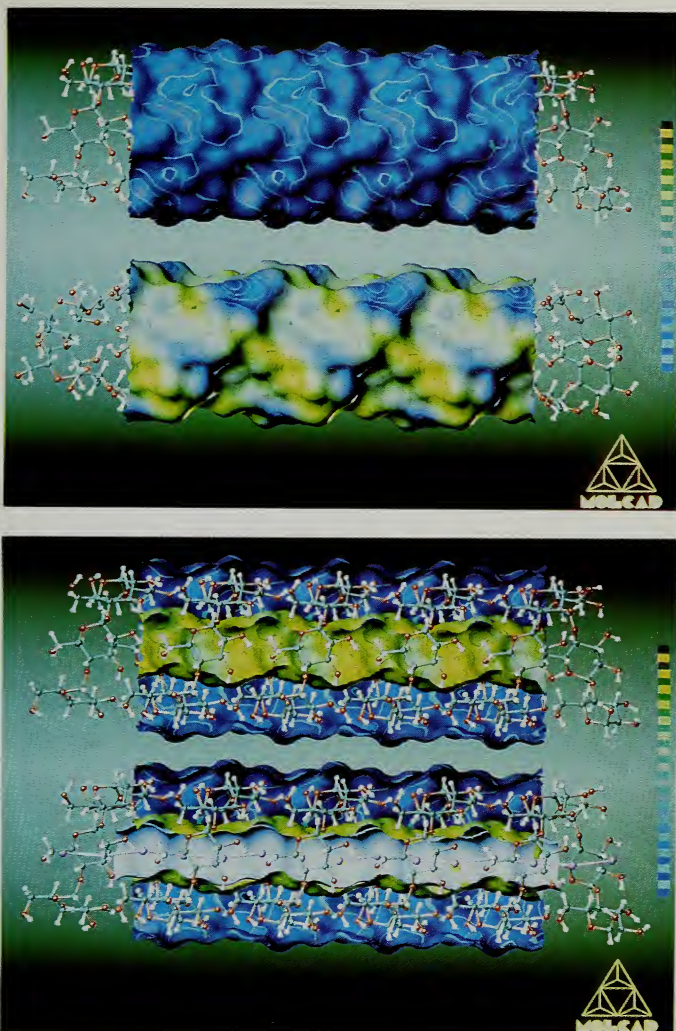


Figure 10. Hydrophobic topographies for the amylose fraction of starch: In the top picture, the MLP pattern on the contact surface of single-stranded V_H -amylose (upper structure) is set against the parallel-stranded double-helical A-form (lower model). In both cases, fragments of approximately 30\AA length are shown, with the surfaces being depicted for the center sections of the rod-shaped polymers only. As is clearly evident, the outside surface area of V_H -type amylose appears to be uniformly hydrophilic, as contrasted to the A-form, which is characterized by an irregular distribution of hydrophilic and hydrophobic regions over the entire surface. Whilst A-starch is a rather compact structure lacking cavities accessible for other molecules, the half-opened model of V_H -amylose (bottom picture) reveals an essentially hydrophobic center channel for this type of starch. It is this almost linear channel which is amenable for the inclusion of fatty-acids by amylose, as well as for the incorporation of iodine/iodide in the well-known deep-blue stained amylose-complex. The nearly perfect linear-chain arrangement of iodine atoms in the pronouncedly hydrophobic environment of the channel is shown in bottommost structure, with the contact surface of the iodine atoms being included in pink-colored form in order to visualize the perfect steric fit.



DISCUSSION

Question: This was an incredible lecture - a most amazingly stimulating lecture. Would you please tell us, to give us some insight, what kind of work is necessary to produce such a model as this. For example, if someone asked you, "Can you make these kinds of models for inulin?," what kind of work do you have to do to produce such a model? How long does it take?

Lichtenthaler: Thank you for this question. A major point is, that this type of modelling can't be done on a standard PC - you have to have a work station, and the proper programs, all coordinated. In the preprint for this paper, which will be handed out after the talk, there are quite a number of references to the programs and machines we used. The work stations are Silicon Graphics work stations; those we used are in the price range of DM 200,000 - they are exceedingly fast. We usually start with the X-ray structure and let it float - that is, we release the motion restrictions of the molecule and let it float to a minimal energy value; these are then the forms we take. In the case of cycloinulin and the cyclodextrins, we took the actual X-ray structures, because these compounds have little flexibility; saccharin, on the other hand, is an entirely rigid molecule. So, its very useful to take solid state structural data, feed them into your computer, via, perhaps, the Cambridge data file, and start the modelling programs. If you have the X-ray structure and are looking for the most probable conformations in solution, as we showed with sucrose, then, of course, more time is required than for modelling isolated molecules in vacuum. If you have the program set and the proper work stations, it's a matter of days or so (computer time) to get some conception of the structure in solution.

Starch molecules are more difficult; as you have seen, we have only modelled amylose because that's the easier part. If you model amylopectin - that is very complicated - it's not a helix, it's not very orderly. We are in the process of doing the additional work to model this.

For standard receptor-substrate interactions, these types of modellings should be an absolute requirement before interpretation of reaction mechanisms. Up to now, we've modelled only substrates - sucrose, sweeteners, etc. What about modelling the receptor? We can get some idea of what the receptor must look like - in geometric as well as hydrophobic/hydrophilic terms - to see where the substrate can fit in. We have some ideas on this, but we're still in the process of modifying and testing. However, this is the direction we're taking - first, substrate modelling; then, with many substrates, a concept of the receptor. Only then you can predict molecular behavior, which is impossible from knowledge of our usual chemical formula alone.

Question: May I add a comment, that in addition to X-ray data alone, it's important to include nmr spectral data, data on structures in solution - perhaps by Raman spectra as Prof. Mathlouthi has shown. One must use as much input data as possible.

Lichtenthaler: We were, of course careful to go into great detail, as have others. We didn't take only the X-ray structure of sucrose to make our concept; we used a great deal of literature data on the structure in solution, such as rotational and NOE-supported NMR data which are more relevant than those from Raman spectra, that do not provide information on intra- and inter-molecular distances. What I meant by "floating" the molecule is the following: we use the X-ray data as a start, and then we let it float and we get an idea of the conformation in solution. Something I've not shown here is, that you can put sucrose in a computer, as a ball into a sea of water, and try to formulate a picture. With sucrose in the middle of a ball (a truncated octahedron actually) filled with water molecules - 571 water in all - we wanted to see how far the sucrose hydrogen-bonding system, whatever it is, influences the water molecules. If you successively take off shells of water around sucrose, down to a monomolecular layer, 8 water molecules remain. One further step down in energy, only 1 water molecule remains. The probability is very high that this last water sits between the glucose 2-oxygen and the primary 1'-OH group of the fructose portion.

A second point - to see the receptor interactions for your modelling, you have to go into certain concentrations where the receptor reacts. The sweetness receptor is much less sensitive than the bitter receptor, by a factor of 600 actually; quinine, for example, can be tasted in a concentration that is 600 times less than a threshold sucrose concentration. Of course, the bitter receptors are on a different part of the tongue, yet the sensitivity difference is quite large. If you model anything, you have to go to concentrations where the taste bud shows a response, and that's a sucrose solution of at least 5% concentration. If you go to lower dilutions - for example, a 0.005% solution, you may not taste anything and I can't say anything about the conformation of sucrose there. In the 5%-10% concentration range where sucrose elicits sweetness, these modellings are very relevant.

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FILTERABILITY OF RAW SUGARS - COMPARING LABORATORY TEST RESULTS WITH REFINERY PERFORMANCE

Michael Donovan and Edward F.T. Lee

Tate & Lyle Ltd., London, United Kingdom

ABSTRACT

The filterability of a raw sugar is one of its key quality parameters, and it has an important effect on refining. As yet we do not have a recognised way of measuring this property in a way that represents filtration performance in a refinery.

A study has been carried out on the filtration characteristics of a number of raw sugars received at Tate & Lyle's Thames Refinery. The filterability of the affined raw sugars has been measured using a method that is a modification to the Nichol森 Horseley filtration test. These modifications are described.

The filtration test has been carried out on both affined raw sugar, and on the melter liquor produced from that raw. A similar filtration test was also carried out on the carbonatation liquor, and all three are compared. Thus filtration characteristics of the raw sugars, and the ways that they behave in the refinery have been studied. The applicability of the test for a carbonatation refinery is discussed.

The test will measure filtration problems caused by suspended solids, but will not indicate whether there will be problems due to starch. Starch will not cause a filtration problem in the test method, but will upset carbonatation, hence causing a filtration problem.

INTRODUCTION

Most refineries use pressure filtration to provide a clear liquor prior to further processing and crystallisation. Filtration is required mainly to provide product quality, but also to facilitate subsequent processing stages such as carbon, char or resin.

Pressure filters are generally used for this application, and they can be used either on their own with filter aid, or after carbonatation, or after phosphatation. As many refineries are run at full capacity a raw that is difficult to filter can cause problems, and certainly Thames Refinery falls into this category.

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There are several ways of overcoming this type of problem; these are to reduce the melt rate, increase filtration temperature, reduce filtration Brix, or increase the lime or filter aid. There are limitations to the extent of the changes that are possible, and Thames Refinery will usually reduce the Brix to cope with poor filtering raws. Reducing the Brix is quite effective in maintaining the filtration rate, because the viscosity is reduced from 12cp to 7cp when the Brix is dropped from 65 to 60. However, the penalty for this is increased energy cost in the form of steam to the evaporator. If the evaporator cannot provide the required Brix increase, then there can be an even greater loss of energy as evaporation in the pan is only equivalent to a single effect evaporator. Feeding lower Brix liquor also causes a loss in capacity as the pans boil slower with a lighter Brix.

The reasons why some raws filter badly have been studied and a number of papers published (1,2,3). Filtration problems are often caused by starch, although this is mainly in carbonatation refineries. The effect of starch on carbonatation has been studied (4), and has been frequently seen and reported by refiners. In many parts of the world raw sugar mills use alphaamylase to keep the starch level fairly low to prevent filtration problems. Nevertheless, some raws are difficult to filter, even with low starch levels, and workers (1, 2, and 3) have tried to identify which other impurities cause filtration problems. There is no relationship between starch and filtration Brix, or dextran and filtration Brix at Thames (5), although as will be described later in this paper, the filtration Brix at Thames is not always a good guide to the extent of filtration problems.

The aim of the present work is to find a laboratory filtration test that can be related to refinery performance, and to establish and quantify the reasons why some raws are difficult to filter. This work is specifically aimed at low starch raws, i.e. raws with starch less than 250ppm.

CHOICE OF A LABORATORY FILTRATION TEST

The most straightforward approach is to set up a standardised filtration test in the laboratory. This test should give a measurement of the filterability of the raw sugar, and it must be representative of the ease or otherwise of filtration in the refinery. As refineries filter affined or washed raw sugar, then the filtration test should be conducted on laboratory affined raw sugar. A test of this sort should be of direct value to a refinery that uses filter aid for filtration, but there were some doubts about whether it could be used to compare with filtration after carbonatation. Thames is a carbonatation refinery, and hence the focus of the work was on this latter point.

We decided to adopt a test similar to that developed by Nicholson and Horsley of CSR in Australia (6), which uses a simple pressure filter, often used in teaching the principles of filtration. We decided to adapt the test to make it more relevant to our use and conditions.

Development of a Laboratory Filtration Test

The Nicholson Horsley test compares test solutions of raw or affined sugar with the filtrate of a pure sugar solution under standard and identical conditions. The standard conditions used are shown in Table 1.

The conditions of this test were changed for the purposes of the Tate & Lyle Sugars work, and are also shown in Table 1. The test is carried out hot and the volume of filtrate for the first 5 minutes collected. The volume of filtrate of a filtered granulated sugar solution, collected at 5 minutes is taken as 100% filterability. The filterability of the sample liquor is represented by the volume of filtrate compared to the volume of filtrate of a filtered granulated sugar solution.

$$\% \text{ Filterability} = \frac{\text{Vol. of Filtrate of Sample}}{\text{Vol. of Filtrate of Filtered Gran. Sugar Soln.}} \times 100$$

A description of the method used for the results presented in this paper is given in Appendix 1. The affination method used to prepare the sugar is described in the Experimental Method section.

The reason that the conditions of the Nicholson Horsley test were changed are given in the Discussion (p. 40). The key point about this test is that in order to express the results in a simple, easy to understand way i.e. a percentage scale, the test must always be conducted over the same time period, 5 minutes in the case of the TLS method. A different result will be obtained if a different time period is used.

EXPERIMENTAL METHOD

Filterability Test of Affined Raw

All the filterability determinations were carried out by the standard laboratory filtration method, and this is described in more detail in Appendix 1. The equipment is essentially a pressure filter and is shown in Figure 1. All of the values of filterability presented in this paper are the average of two tests.

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Affination of Raw Sugar

Transfer 3000g raw sugar to 450 ml of saturated granular sugar solution.

Stir for 10 minutes at 100 rpm.

Centrifuge at 3000 rpm for 2 minutes.

Remove sugar from basket and dry at about 90°C.

Cake Resistance of Carbonatated Liquor

The filtration equipment shown in Figure 1 was also used to measure the cake resistance of the carbonatated liquor. The method is also carried out at 70°C and 60 psi. The following basic constant pressure filtration equation was used to evaluate the cake resistance:

where t : filtration time (sec)
 V : volume of filtrate (cm^3)
 r : cake resistance (cm^2)
 μ : filtrate viscosity (poise)
 v : volume of cake deposited per unit volume of filtrate
 A : filter area (cm^2)
 ΔP : applied pressure (dynes/cm^2)

A plot of t/V vs V will give a straight line and the cake resistance can be calculated from the slope.

All of the values of cake resistance presented here are the average of two tests.

Suspended Solids

The suspended solids were separated by centrifugation at 30,000g from a 14 Bx solution. This was carried out with a high speed centrifuge at 17,000 rpm for half an hour at room temperature. The suspended solids were then well washed with distilled water and filtered through a 0.22 μm membrane filter (Millipore GVWP). The solids recovered were dried at 105°C and weighed. The ppm value was based on total solids as measured by Brix.

Turbidity

This was measured at 900 nm by a PYE UNICAM UV/VIS Spectrophotometer. The sample solution was diluted to 14 Bx and measured in a 1 cm cell. This calculation used was:

$$\text{Turbidity} = 1000 \times \text{Absorbance} / \text{Brix}$$

COMPARISON WITH REFINERY

The work was started by collecting samples of raw sugar received at Thames Refinery, measuring the filterability of the affined raw in the laboratory, and collecting as much data and samples as possible from the refinery when those raws were processed. The samples and readings collected represented 24 hour averages. One problem was that Thames often mixes raws, and hence data and samples were only collected when the specific raw represented at least 80% of the melt. The main samples collected, other than the raw sugar, were a sample of the melter liquor, and a sample of the carbonatated liquor, i.e. after carbonatation with the calcium carbonate still in the liquor. About 50 raws were studied in this way.

The first approach was to attempt to correlate the affined raw filterability to the filter press station performance at Thames Refinery. The pressed liquor Brix was chosen to represent the ease of filterability of the carbonatated liquor, because when filtration problems occur at Thames the Brix is lowered. Figure 2 shows a graph of the filterability of the affined raw and the pressed liquor Brix at Thames Refinery. There is a general trend that the filterability of the laboratory affined raw decreases with decreasing pressed liquor Brix, but the relationship is not very good.

Figure 2 suggests that either the laboratory test does not represent the filterability in Thames Refinery, or that the pressed liquor Brix does not fully reflect filtration problems. The Brix is often changed for reasons that are not related to filtration problems, but it was hoped that for this work with the large number of raws studied there would still be a correlation. It was therefore decided to investigate the situation further. We had also collected other samples and data in case we had a problem relating raw sugar filterability to pressed liquor Brix.

There are two sampling points where laboratory filtration can be used to measure filterability in the refinery. These are the melter liquor and the carbonatated liquor, as shown in Figure 3. The melter liquor filterability will represent the filterability of the refinery affined raw, plus that of other materials added to the melter. The cake resistance of the carbonatated liquor will reflect the filtration

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resistance of the melter liquor after carbonatation. This filtration carried out in the laboratory is on the same liquor that is filtered in the refinery on the Sweetland presses.

It is difficult to collect refinery affined sugar at Thames because this is dropped straight from the centrifuge into water in the scroll feeding the first melter.

Figure 4 shows the relationship between the filterabilities of the laboratory affined raw and the corresponding melter liquor at Thames Refinery. This gives $R^2 = 0.85$. R^2 is the square of the correlation coefficient, representing the proportion of variability explained. There is some scatter of the data and it shows that the melter liquor has a lower filterability than that of affined raw. This is probably because the laboratory affination is more efficient than refinery affination. Also the melter liquor can contain a portion of other raws mixed in from raw sugar stocks, plus first crop sugar from the recovery house melter.

The filterability of the carbonatated liquor was assessed by adapting the filterability term $1/\sqrt{r \times \text{CaO}}$ from the work of Bennett (7,8), where r is the specific cake resistance and CaO is the lime dosage. Figure 5 gives the relationship between the melter liquor filterability and the corresponding modified carbonatated cake resistance term $1/\sqrt{r \times \text{CaO}}$. The number of data points has been reduced, choosing only those points with a final pH between 7.9 and 8.6. The carbonatation plant over the period when the data and samples were collected was in a poor state, and has subsequently been replaced. As a consequence there were a lot of problems with lime dosing rate, gas flow and pH control. The final value of pH is very important in carbonatation, and our results showed that pH outside the range of 7.9 to 8.6 gave a wide variation in filterability, confusing the analysis, but this is probably to be expected. The new plant has much better final pH control.

The data in Figure 5 gives $R^2 = 0.82$ and the statistical analysis indicates that the correlation is highly significant. A similar graph can be plotted of laboratory affined raw filterability verses carbonatation cake resistance factor, shown in Figure 6, and this has an R^2 of 0.81.

The relationship between melter filterability and the cake resistance after carbonatation demonstrates that the laboratory filterability test can be used to represent the filterability of a raw sugar in a refinery. In view of this the test was used to study a range of raw sugars to find out why some filter better than others.

REMOVAL OF SUSPENDED SOLIDS

The most likely reason for filtration problems is the presence of suspended solids, and suspended solids were the only impurity to relate to filterability in the work of Devereux and Clarke (2). We therefore carried out a series of simple tests to remove the suspended solids, and then re-measured the filterabilities of several raws.

Table 2 shows the filterabilities of a number of laboratory affined sugar solutions, and their corresponding filtrates, i.e. they were filtered twice through the filtration test equipment, and filterabilities measured both times. It can be seen that the filterability of the filtrates are far higher than those of the original solutions. This indicates that the majority of the filtration impedance was removed by the first filtration, and this is almost certainly suspended solids. It is interesting to note that the filtrates did not have a filterability of 100%, i.e. equivalent to dissolved granulated sugar, but as the filterability scale is not linear the values all represent good filtering liquors.

The filterability data indicate that suspended solids are the main filtration resistance rather than the viscosity.

Suspended solids were also removed by centrifugation, and once again the filterability was much better after their removal. This is shown in Table 3. In this case a range of 7 liquors were centrifuged using the method described in the Experimental Section.

SIZE AND SIZE DISTRIBUTION OF SUSPENDED SOLIDS

Screening

It was decided to investigate the size of the particles in affined raw sugar as this may have an effect on filterability. It has been observed that affined sugar solutions contain large particles that settle quickly, and a 24 hour settling period was used to remove these. Other particles were removed by sequential screening through metal screens down to 5 microns aperture size. Table 4 gives the filterability of an affined sugar solution (raw sugar sample A) after such screenings.

Particle Size Analysis

It is shown in Table 4 that the suspended solids that cause filtration problems in the raw sugar sample A are less than 5 microns. Work was carried out in an outside laboratory using a Malvern Mastersizer, and the particle size data is shown in Figure 7. This is a volume distribution of the sample that has been screened through 10 microns. This particle size analysis has revealed that the sample had particles

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with peaks around 0.5 μm and 2.5 μm with the higher proportion in the first peak.

EFFECT OF SUSPENDED SOLIDS ON FILTERABILITY

Figure 8 shows the effect of suspended solids on filterability of a number of raw sugars from a wide geographical spread. The suspended solids have been measured using the technique described in the Experimental Method section, i.e. they have been separated by centrifugation. These are not only raws received at Thames refinery, but raws collected from around the Tate & Lyle Group companies. There are also some factory A and B sugars, the B sugars showing the lowest values of filterability.

Figure 9 shows the relationship of filterability to suspended solids for those raw sugars delivered and processed at Thames Refinery. These are a sub-set of the data in Figure 8.

TURBIDITY

Turbidity can often be used to indicate suspended solids. The turbidity of a number of the samples was measured using the method indicated in the Experimental Section. Measuring turbidity is a simpler way of assessing suspended solids. Figure 10 shows the effect of turbidity on filterability, and the filterability was also found to get worse with increasing turbidity.

DISCUSSION OF RESULTS

It is important that a laboratory filterability test gives results that relate to refinery performance. For a carbonation refinery this means that the test must give results that can be related to the filtration of the cloudy liquor after carbonation. The test itself must be carried out on a sample of the raw sugar as received to allow a view to be formed on why some raws filter better than others. As mentioned earlier it is also important that the test be carried out on affined raw sugar, because it is affined sugar that is sent to the melter to be processed. The majority of the syrup film on the raw sugar surface is sent to recovery where filtration is not usually an issue.

This work has used a laboratory pressure filtration test based on that developed by Nicholson and Horsley (6). The conditions for the Nicholson Horsley test were changed to give a better match to refining conditions. The temperature was changed from ambient to 70°C because this is the filtration temperature in the refinery, and some materials may be insoluble at room temperature, and dissolve when heated

affecting filterability. It was also felt that there were problems running the test at pH9, because of precipitation of phosphate when the pH is raised, again affecting filterability. Hence the test was carried out at natural pH, usually about pH6.5. The filter paper was changed because inconsistent results were found with Whatman No. 1, and these were resolved when Whatman GFA was used.

This work was aimed at low starch raws where the level of starch does not significantly affect carbonatation and filtration of the subsequent cake. Using the results from 50 raw sugars the filterability of the affined raw showed a relationship with the Brix of the filtration liquor i.e. pressed liquor Brix, but as shown in Figure 2 there is a lot of scatter. Because of this the other samples collected were tested for filterability, to find out where this scatter was introduced. The other samples were taken from the points shown in Figure 3, melter liquor which represents all of the materials added to the melter, and carbonatation liquor, which is the calcium carbonate slurry after carbonatation. The latter is the stream that is filtered on the Sweetland presses, and the filtration of this stream should match the ease or otherwise of filtration in the refinery.

Figure 4 shows a good correlation between affined raw sugar and melter liquor filterabilities. The differences between the two samples could be due to:

- a) the melter may only contain 80% of the specified raw, due to blending of raws
- b) the melter has refinery affined sugar, which is not as complete as laboratory affination.
- c) other streams, mainly the recovery melter stream are added to the main melter.

Despite the above the relationship is good enough to conclude that the scatter is not introduced at this stage.

Figure 5 shows the filtration characteristics before and after refinery carbonatation. The filterability of the melter liquor is straightforward. After carbonatation the cake resistance is measured, but to allow for the variation in the amount of lime used, a term using both cake resistance and lime dosage is used. Another modification to the data was necessary due to the poor state of the carbonatation plant. Because of control difficulties the final pH varied over a wide range, and it is known that this can have a large effect on filtration characteristics. The aim of the work was to assess the filtration characteristics after properly controlled carbonatation, and hence only the data collected from the correct pH range of pH7.9 to pH8.6 was used. This resulted in a quite reasonable correlation between filtration characteristics before and after carbonatation,

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shown in Figure 5. The R^2 value was 0.82, showing that the filterability test does provide a laboratory method of predicting filtration performance. There is also an R^2 value of 0.81 for the filterability of affined raw sugar with filtration after carbonatation, Figure 6. Hence a sample of raw can be taken from the shed or from the ship and tested to assess its filtration characteristics in the refinery providing the starch level is low. If the starch level is high, i.e. 250ppm or more we would not expect this relationship to stand, and more work is required in this area.

Filtration problems can be caused by a number of things, among them suspended solids and an increase in viscosity. The simple test carried out of filtering out the suspended solids and measuring the filtration again, as shown in Table 2 shows that the main problem is suspended solids. Similarly centrifuging out the suspended solids gives a big improvement in filterability, as shown in Table 3. These suspended solids are very fine, Table 4 shows that screening out particles over 5 microns had very little effect on filtration. Further investigation using a Malvern particle size analysis showed that many of the particles are sub-micron, Figure 7.

The centrifugation method described in the Experimental Section will remove and quantify the suspended solids causing the filtration problem. After centrifugation the liquors filtered quite well, and this method was used to quantify the amount of suspended solids. A large number of raw sugars were collected from many countries of origin and Figure 8 shows that increase in the suspended solids level reduces filterability. At Thames we would like to receive raw sugars that when affined have filterabilities of 50% or more, and this means a suspended solids level of no more than about 300ppm.

Less data has been collected on turbidity, and more work is planned. Nevertheless, Figure 10 shows that turbidity at 900nm also gives a good guide to filterability, and using the 50% affined raw filterability target, turbidities less than 200 would give good filtration characteristics at Thames.

CONCLUSION

A laboratory filterability test has been developed that can be used on a sample of raw sugar to predict whether that sugar will filter well or badly at Thames Refinery. It is based on a well known technique developed by Nicholson and Horsley at CSR in Australia, but modified to match refinery conditions more closely. This test will predict filtration after carbonatation, but we believe that this is only the case with low starch raws, because it is well known that starch will affect the formation of calcium carbonate, causing filtration problems.

The test was used to show that the filtration problem is caused by suspended solids, and that these are very fine particles, mainly sub-micron. Levels of suspended solids greater than 250ppm will reduce the filtration ability of Thames refinery. Turbidity also relates to filterability and turbidities greater than 200, measured at 900nm, will also cause filtration problems.

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APPENDIX 1

Standard Method Procedure

1. Prepare sample solution and adjust to 65 ± 0.05 Bx in a plastic bottle. Add $0.5\% \pm 0.002\%$ (on solid) Celite 505, stopper and heat in 72°C water bath for 45 minutes with occasional shaking.
2. Assemble pressure filtration unit, mount in jacket and attach an outlet valve to the outlet of the filter unit in a closed position.
3. Circulate water through jacket and maintain at 70°C , check for leakage of filtration unit.
4. Transfer sample to the filter through a funnel, start stop watch simultaneously and allow to stand for 3 minutes before filtration.
5. Connect filter to the pressure supply cylinder when 2.75 minutes has elapsed.
6. At 3 minutes, raise the applied pressure instantly (pre set at 60 psi) and simultaneously open the stop valve.
7. Collect filtrate in a measuring cylinder. Record volume and time.
8. Finally, mix filtrate, cool and measure Brix to check leakage.
9. The volume of the filtrate collected at 5 minutes is used to calculate filterability.

$$\% \text{ Filterability} = \frac{\text{Vol. of Filtrate of Sample}}{\text{Vol. of Filtrate of Filtered Gran. Sugar Soln.}} \times 100$$

Standardisation of Filterability

1. Prepare a granulated sugar solution and adjust to 65 ± 0.05 Brix.
2. Add $0.5\% \pm 0.002\%$ (on solid) Celite 505, heat in 72°C water bath for 45 minutes and filter at 70°C with Whatman GFA paper.
3. The filtrate is then used for standardisation. Proceed as described in Standard Method.

4. The filterability of a filtered granulated sugar solution is taken at 100%.

Table 1. Nicholson Horsley and Tate & Lyle Sugars Filtration Tests

	Nicholson Horsley	Tate & Lyle Sugars
Liquor	60 Bx	65 Bx
pH	9.0	Natural (Ave. is pH6.5)
Temperature	Room	70°C
Pressure	50 psi	60 psi
Filter Paper	Whatman No. 1	Whatman GFA
Filter Aid 0.5%	Celite 505	Celite 505
Liquor pre-heat	-	45 minutes

Table 2. Filterabilities of Affined Sugar Solution and Corresponding Filtrates

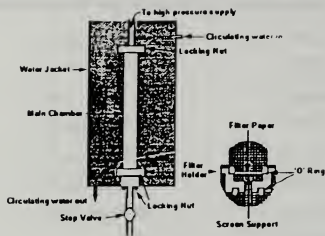
Sample	Filterability %	
	First Filtration	Second Filtration
Affined Sugar Solution 1	55.5	81.5
Affined Sugar Solution 2	68.4	91.1
Affined Sugar Solution 3	64.2	87.3
Affined Sugar Solution 4	49.4	91.2
Affined Sugar Solution 5	50.4	79.4
Affined Sugar Solution 6	54.3	89.8
Affined Sugar Solution 7	37.4	76.3
Affined Sugar Solution 8	36.2	73.7
Affined Sugar Solution 9	40.2	81.0
Affined Sugar Solution 10	39.4	76.6

Table 3. Filterabilities of Affined Sugar Solutions and Supernatants

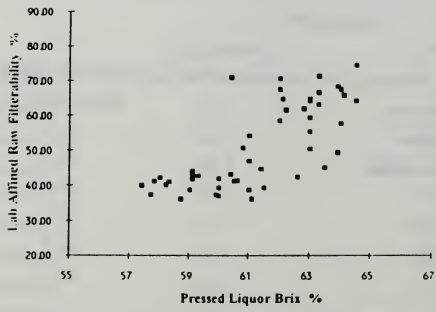
Syrup Sample	Liquor F%	Supernatant F%
Sample 1	37.0	77.2
Sample 2	44.3	81.1
Sample 3	53.9	73.4
Sample 4	47.6	73.8
Sample 5	53.4	79.9
Sample 6	50.3	82.3
Sample 7	59.5	73.6

Table 4. Filterability of Screened Affined Sugar Solution Sample A

Screen μm	Filterability %
Original	45.1
24 hours settling	45.4
38	46.6
20	45.8
10	46.4
5	46.1



**FIG 1 DIAGRAM OF FILTRATION
TEST EQUIPMENT**



**FIG 2 LAB AFFINED RAW FILTERABILITY
VS PRESSED LIQUOR BRIX**
(Thames Refinery)

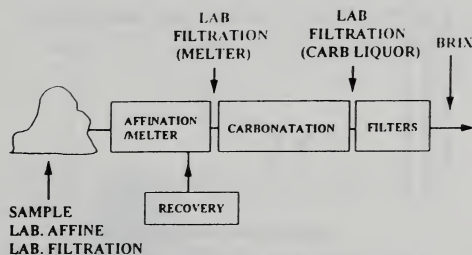
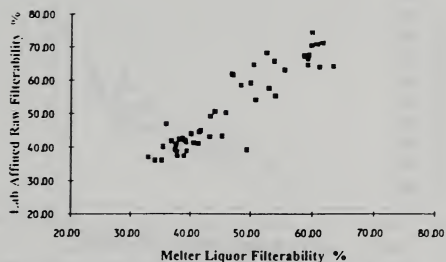
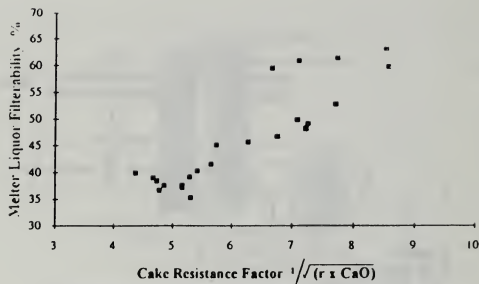


FIG 3 SAMPLING POINTS



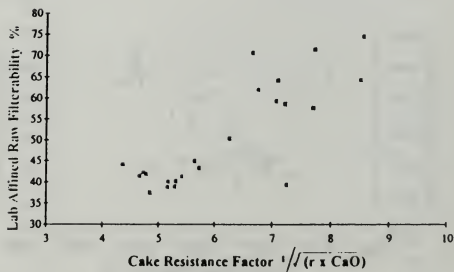
**FIG 4 LAB AFFINED RAW FILTERABILITY
VS MELTER LIQUOR FILTERABILITY**

(Thames Refinery)



**FIG 5 MELTER LIQUOR FILTERABILITY
VS CAKE RESISTANCE FACTOR**

(Thames Refinery)



**FIG 6 LAB AFFINED RAW FILTERABILITY
VS CAKE RESISTANCE FACTOR**

(Thames Refinery)

1994

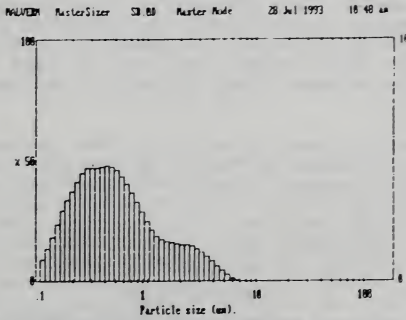


FIG 7 PARTICLE SIZE ANALYSIS
(By Volume)

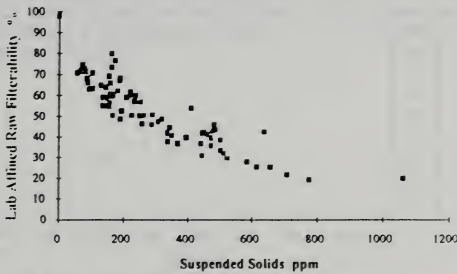


FIG 8 LAB AFFINED RAW FILTERABILITY
VS SUSPENDED SOLIDS
(All Raws)

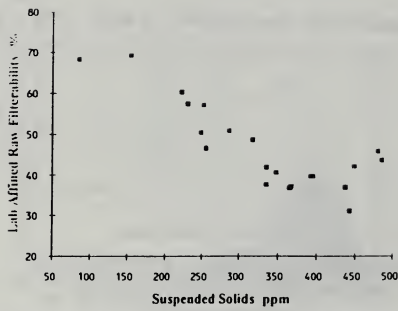


FIG 9 LAB AFFINED RAW FILTERABILITY
VS SUSPENDED SOLIDS
(Thames Refinery Raws)

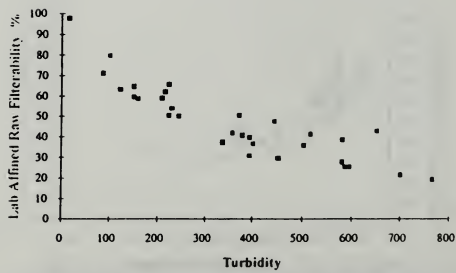


FIG 10 LAB AFFINED RAW FILTERABILITY
VS TURBIDITY
(All Raws)

DISCUSSION

Question: Thank you for a good demonstration of how to use a refinery to prove research results. Your conclusions are valuable. I want to make a couple of suggestions about how you can use your tests in a practical way: by heating your affined sugar solution until the starch is dissolved, you could do a second filtration and determine the effect of starch on filtration in the same test. This would give an idea of how the starch level would affect the process, because the viscosity would develop as the starch is dissolved.

A further comment on SPRI's 1984 work (your reference no. 2) which showed that suspended solids were often responsible for filtration problems in what you call "low starch" sugars: we investigated the composition of the suspended solids, and found that most of the material was field soil in origin, and that particles of diameter 2μ or less were in the critical size range that blocked filters. The field soil was identified because the filter-blocking material contained aluminum, a soil element which sugarcane does not absorb, thereby identifying the blocking material as coming from soil, not from cane. That composition factor might provide another test for filter blocking material. This is a suggestion for your future work.

Donovan: We have in fact been looking at the composition of the suspended solids. In actual fact, we thought it would be all inorganic, but it's not, it's part organic. The inorganic has a lot of silicon content, it has aluminum, some iron and calcium. We haven't really been able to establish a good pattern yet. We've done some work on the organics, but can't yet conclusively say what the organic material is.

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SEPARATION AND CHARACTERISATION OF POLYSACCHARIDES FROM SUGARCANE
IMPURITIES: USE OF NMR TECHNIQUES

Riaz Khan, Antonella Meliota, Erminio Murano, and Renato Toffanin,
POLY-tech, Area di Ricerca, Padriciano 99, Trieste, Italy

ABSTRACT

Sugar refining involves successive processes for removing a variety of impurities. The nature of the impurities such as charge, hydrophobicity and hydrophilicity, and molecular weight bear considerable influence on the purification process. As part of a larger programme on sugar refining we are currently investigating the impurities, in particular high molecular weight impurities, present in the melter liquor.

The high molecular weight compounds, polysaccharides, have been isolated by methanol precipitation and further fractionated using gel permeation chromatography. The structure and molecular weight of these polysaccharides have been determined by conventional nuclear magnetic resonance (nmr), laser light-scattering spectrometer, and by high performance gel permeation chromatography. The oligosaccharides obtained by enzymic hydrolysis have been studied using advanced nmr techniques based on selective excitation experiments. In particular, 1D TOCSY experiments have been performed to assign the individual spin systems and 1D NOESY experiments to locate the branched glucose residues.

INTRODUCTION

Clarke and co-workers have isolated and investigated the structure and influence of polysaccharides present in high molecular weight cane sugar colorants (1,2,3). Raw cane sugar contains about 0.1 to 0.2% polysaccharides. It has been suggested that polysaccharides may act as carrier molecules for the transfer of high molecular weight colorants into the crystal where they become trapped and they diffuse out with difficulty (4).

In this communication we describe our preliminary results for the separation of sugarcane polysaccharides and their characterisation by nmr and gel permeation chromatography.

RESULTS AND DISCUSSION

Isolation of Polysaccharides

The affinated syrup sample was precipitated by addition of methanol to a final concentration of 75%. The precipitate formed was collected by centrifugation and washed with 75% methanol to afford the total solid residue of 0.12%. This fraction was then suspended in 0.05 M potassium phosphate buffer (pH 6.8), centrifuged to remove the insoluble material (71%), and the supernatant (29%) was subjected to fractionation by gel permeation chromatography (GPC).

The GPC separation was achieved on a HiLoad Superdex 200 (2.6 x 60 cm) prep. grade column (Pharmacia) equipped with a UV (280 nm) and refractive index detectors. The column was eluted with 0.05 M potassium phosphate buffer (pH 6.8.), sample volume 5 ml, flow rate 55 ml/h, at 25°C. Most of the material was eluted in the void volume and close to the total volume fractions. Three main components I, II and III were obtained in yields of 49, 13 and 38% respectively (Figure 1).

Molecular Weight Determination

The molecular weight averages and the molecular weight distribution of the above fractions were determined by high performance size exclusion chromatography (HPSEC) system (5). The signals were detected using an LDC-chromatix CMX-100 low angle laser light scattering (LLALS) photometer (equipped with a He-Ne laser, $\lambda_0 = 632.8$ nm) and a Waters 410 differential refractometer. The column was set (Toso Haas TSK, G6000 + G5000 + G3000 + Guard column) at 40°C and HPLC Jasco 980 PU pump was used with a flow rate of 0.8 ml/min. The injector was Rheodyne 9125 with a 100 μ L loop. Sodium chloride (0.1M) (0.22 μ m Millipore filters) was employed as an eluant and the calibration was performed using dextrans as standards (WHO, 40,000-2,000,000). All samples (1g/L) were filtered through 0.45 μ m Millipore filters before the injection.

The molecular weight (2,600,000) of fraction I and its distribution (Table 1) were similar to those of the commercial dextran 2000. Fraction II (MW 131,000) was found to be heterogeneous, and fraction III had a MW 16,000 with a polydispersity index (P.I.) of 1.4.

Enzymic Hydrolysis

Enzymic hydrolysis was performed with the compounds I and III. In a typical experiment the compound (50 mg) in distilled water was treated with 500 units of dextranase (Sigma from a Penicillium sp.), at pH 5.5 at 39°C for 24 hr, followed by two further treatments with the enzyme, 500 units each, at an interval of 24

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hr. The reaction was then stopped by heating at 100°C for 5 min. and the solution was freeze dried. The hydrolysed samples were then subjected to size exclusion chromatography on a Sephadex G25 Super Fine (Pharmacia) column (59 cm x 2.5 cm), using distilled water as the eluant. Compound I led to four fractions, the void volume presumably containing the unhydrolysed or partially hydrolysed starting material IA (dextran), IB and IC oligosaccharides, and isomaltose or glucose ID in the total volume (Figure 2). Similarly, compound III on further fractionation gave four major fractions, IIIA in the void volume, probably containing the starting material, IIIB, IIIC, and then a major peak IIID in the total volume (Figure 3).

Nmr Analysis

¹H and ¹³C nmr experiments were recorded in D₂O on a Bruker AC 200 nmr spectrometer equipped with a 5 mm broad-band probe. ¹H chemical shifts were measured in parts per million from internal sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propionate (TSP). Proton composite pulse decoupled ¹³C nmr spectra (50.33 MHz) were recorded overnight and chemical shifts were referred to trimethylsilane (TMS) by setting the internal dioxan at 67.4 ppm.

Two-dimensional homonuclear COSY and relayed COSY data were acquired using 512 and 256 data points in F₂ and F₁, respectively, with zero-filling in F₁. Centered sine-bell weighting was applied prior to Fourier transformation.

1D TOCSY and 1D NOESY spectra (6) were recorded using the decoupler as the sole source of ¹H RF pulses and applying DANTE pulse trains (7) for a selective spin-echo excitation (8). In 1D TOCSY experiments the spin-lock consisted of the MLEV-17 pulse sequence sandwiched between two trim pulses (9). For 1D NOESY spectra two values of mixing time (τ_m , 600 and 800 ms) were used.

The ¹³C nmr spectrum of fraction I (Figure 4) showed a pattern identical to that of a standard, essentially unbranched dextran (2). The characteristic signals of α (1-6) linked glucose present in a linear dextran, were identified in the ¹³C spectrum of fraction III (Figure 4) together with additional signals attributed to branched glucose units. Integration of the anomeric signals of the branched (100.5 ppm) and linear dextran (98.5 ppm) indicated that the degree of branching was approximately 70%. Similarly, the ¹H-nmr spectrum of I (Figure 5) showed only one signal at 5.00 ppm, in the anomeric region, whereas in the ¹H spectrum of III (Figure 5) there was an additional signal at about 5.4 ppm which was attributed to the anomeric resonances of the branched glucose residues.

The ^{13}C spectrum of fraction II (Figure 4) revealed the presence of two different patterns. One pattern was attributed to an arabinogalactan according to Clarke et al. (2), with α -D-arabinofuranose and β -D-galactopyranose in the ratio of 7 to 3, while the other pattern was ascribed to the unbranched dextran found in fraction I. Integration of the ^1H anomeric signals of arabinogalactan (5.25 and 5.10 ppm) and dextran (5.00 ppm) revealed that the ratio of the two polysaccharides were approximately 1:1.

The ^1H nmr spectrum of the oligosaccharidic fraction I-D (Figure 6) showed three anomeric signals at 5.29, 5.00 and 4.73 ppm attributed to isomaltose and these assignments were confirmed by ^{13}C nmr data. The same three signals were also observed in the anomeric region of the ^1H spectrum of III-D together with a new signal at 5.45 ppm (Figure 6), which was attributed to H-1 of a branched glucose unit. Fraction III-D was then subjected to detailed nmr analysis and a series of homonuclear 2D correlation spectroscopy (COSY) experiments were performed for unambiguous assignment. Initially, a basic COSY experiment was performed on III-D to correlate directly coupled protons. Subsequently, one-step and two-step relayed COSY spectra were recorded to correlate protons not directly coupled. The one-step relayed correlation spectrum of III-D with cross peaks indicating the direct connectivities between H-1's and H-2's, and the relayed connectivities between H-1's and H-3's of the different glucose units (Figure 7). Homonuclear 2D correlation experiments allowed the assignment of most of the ^1H -nmr signals, however, for a better characterisation of the oligosaccharide structure, selective 1D nmr spectra were recorded. In particular, 1D TOCSY experiments were performed with different mixing times to unravel the individual spin systems and to confirm the previous assignments. As shown in the 200-MHz 1D TOCSY spectrum (τ_m 120 ms) of III-D (Figure 8), the selective irradiation of H-1' at 5.00 ppm clearly revealed only the resonances due to H-2', H-3' and H-4' of the α (1-6) linked glucose residues.

Finally, a series of 1D NOESY experiments was recorded to locate the branched glucose residue in III-D. In the 1D NOESY spectrum, obtained by selective irradiation of Glc, H-1" at 5.45 ppm, two signals were present in the region 3.9-3.4 ppm (Figure 9). One signal at about 3.6 ppm was assigned to Glc, H-2" (1D COSY signal), whereas the other at about 3.7 ppm, although a little distorted, was attributed to Glc, H-3 α . These data clearly indicate that the branched residue is located on position 3 of the Glc, unit at the reducing end, thus confirming the previously reported data on the linkage site in branched dextrans (10).

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Table 1. Molecular weight distribution.

Fraction	Peak I		P.I.	Peak II		P.I.	Peak III		P.I.
	Mw	Mn		Mw	Mn		Mw	Mn	
I	260000	114500	2.3						
II	252500	140000	1.8	131000	111000	1.2			
III							16000	12000	1.4

Chromatographic system (HPSEC):

Pump: HPLC Jasco 980 PU (Flow rate: 0.8 ml/min)

Injector: Rhodyne 9125 with a 100 μ L loop

Columns: TosoHaas TSK (G6000 + G5000 + G3000 Guard column); T=40°C

Detectors: RI410 Waters (Sens x 128; T=35°C); LALLS Chromatix CMX 100

Eluant: NaCl 0.1M (0.22 μ m Millipore filters)

Calibration was performed using dextrans as standards (40,000-2,000,000)

Samples were filtered through 0.45 μ m Millipore filters just before injection.

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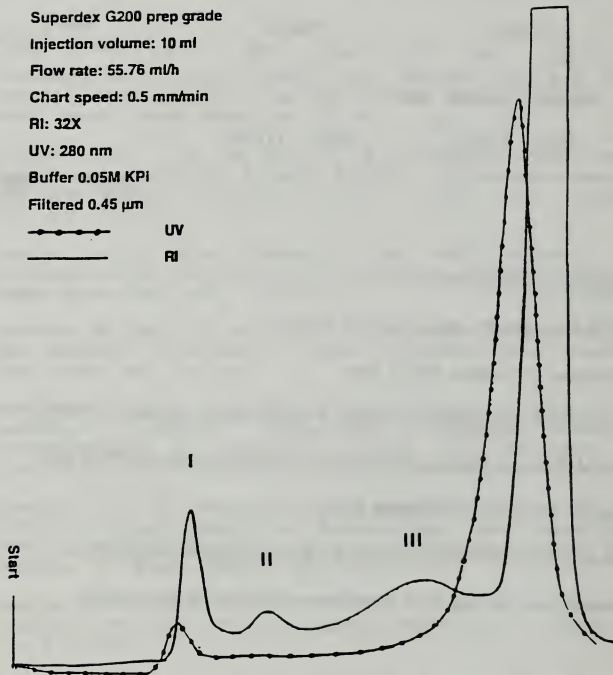


Figure 1. GPC on Pharmacia Superdex 200 (2.6 x 60 cm) equipped with a UV (280 nm) and refractive index detector. The solvent is 0.05 M potassium phosphate buffer, pH 6.8, and separation is performed at room temperature.

Sample : Fraction I after enzymic hydrolysis

Column : Sephadex G-25 Superfine (Pharmacia) 59 X 2.5 cm

Eluent : distilled water

Pump : 3

Flow rate : 18.58 ml/h

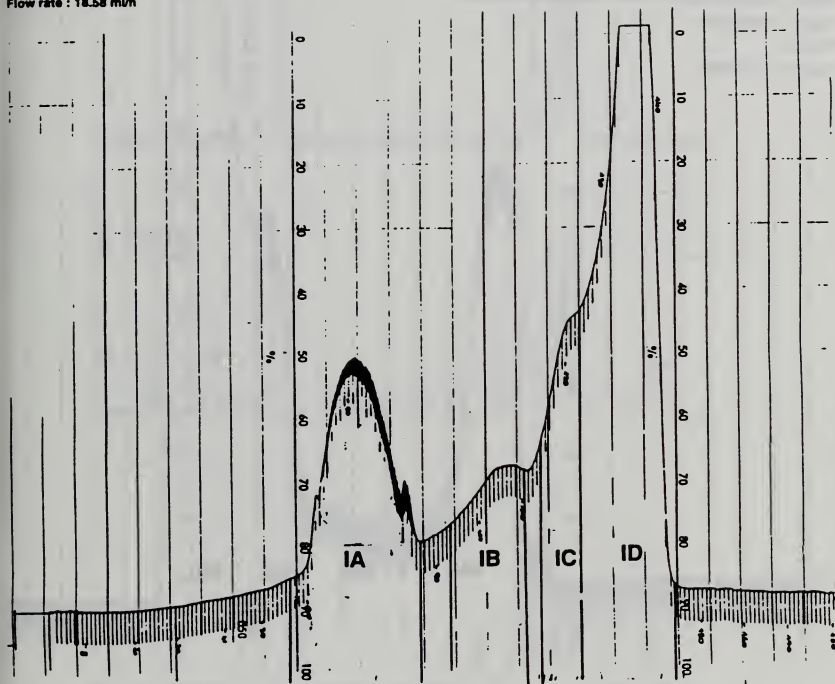


Figure 2. Fractionation of I on Sephadex G-25 (Pharmacia)

Sample : Fraction III after enzymic hydrolysis

Column : Sephadex G-25 Superfine (Pharmacia) 59 X 2.5 cm

Eluent : distilled water

Pump : 3

Flow rate : 18.58 ml/h

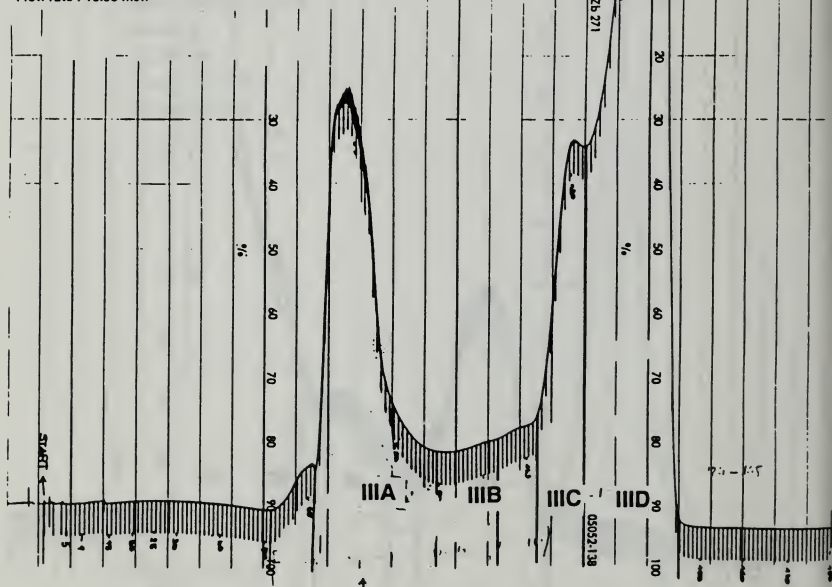


Figure 3. Fractionation of III on Sephadex G-25 (Pharmacia)

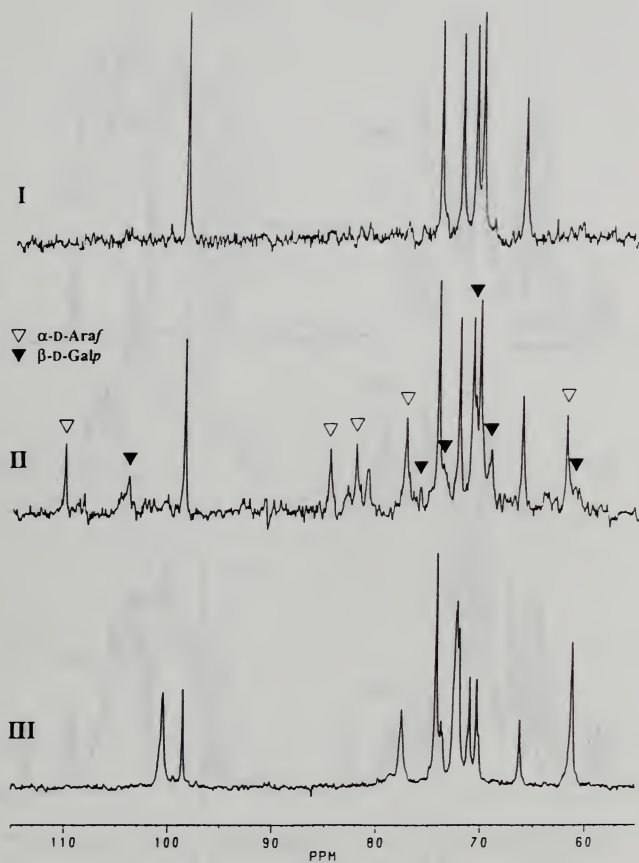


Figure 4. 50.3 MHz ^{13}C n.m.r. spectra of fractions I, II and III.

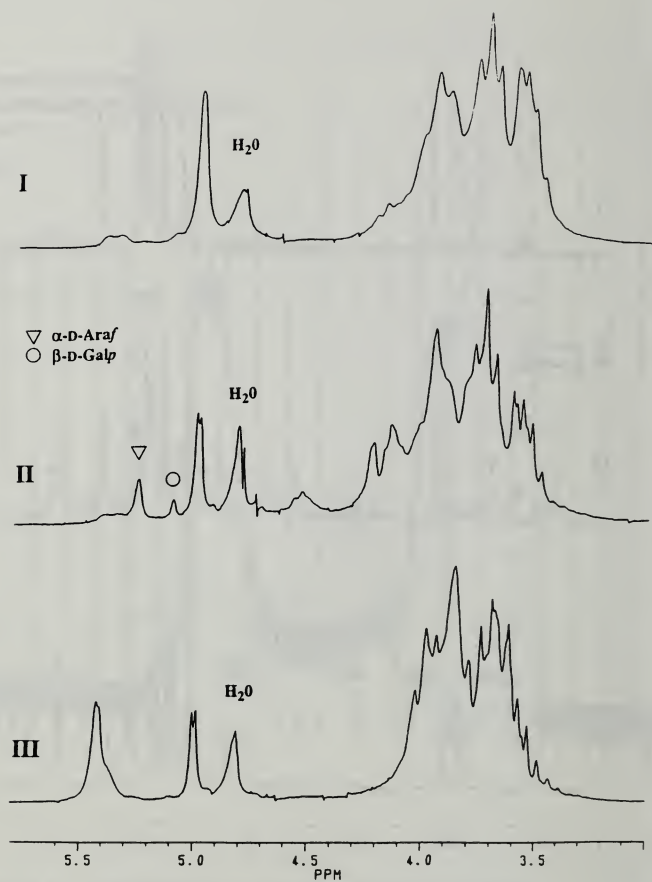


Figure 5. 200-MHz 1H nmr spectra of fractions I, II and III.

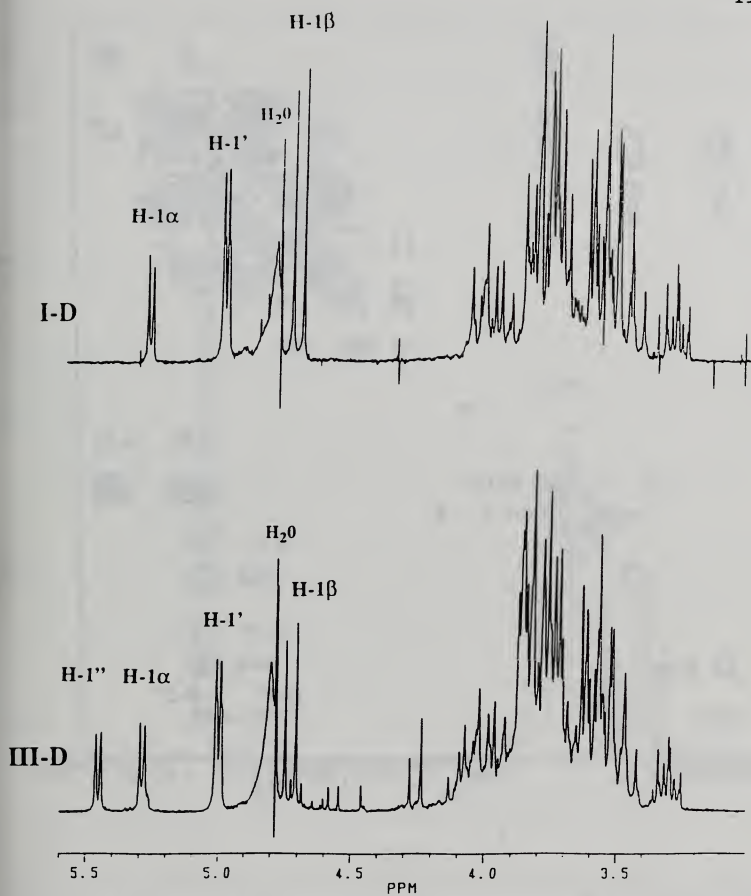


Figure 6. 200-MHz ¹H nmr spectra of fractions I-D and III-D.

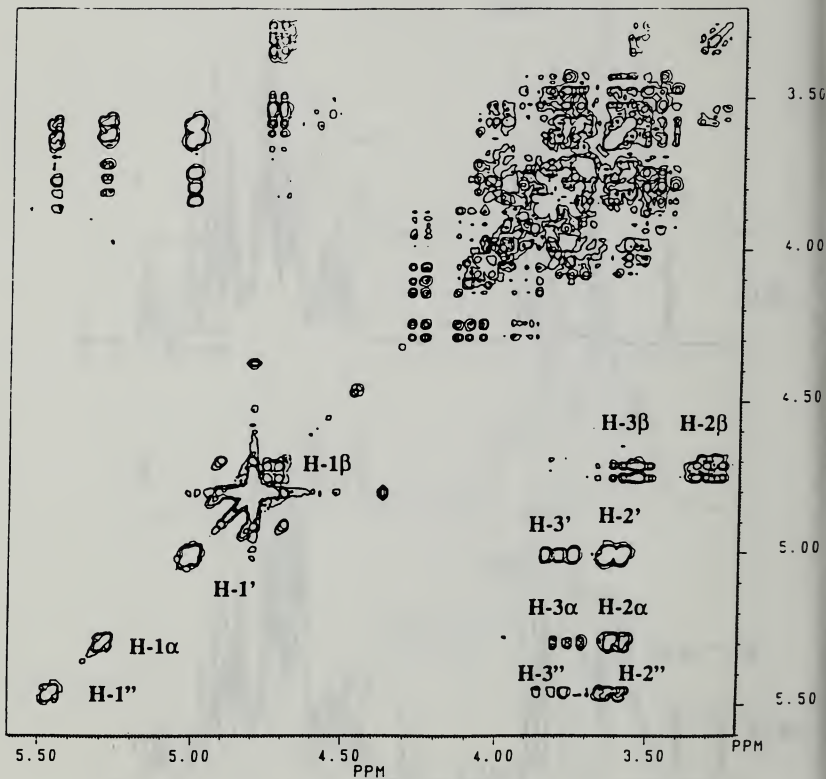


Figure 7. 200 MHz ^1H - ^1H relayed COSY contour plot of fraction III-D.

H-1'

H-3'

H-4'

H-2'

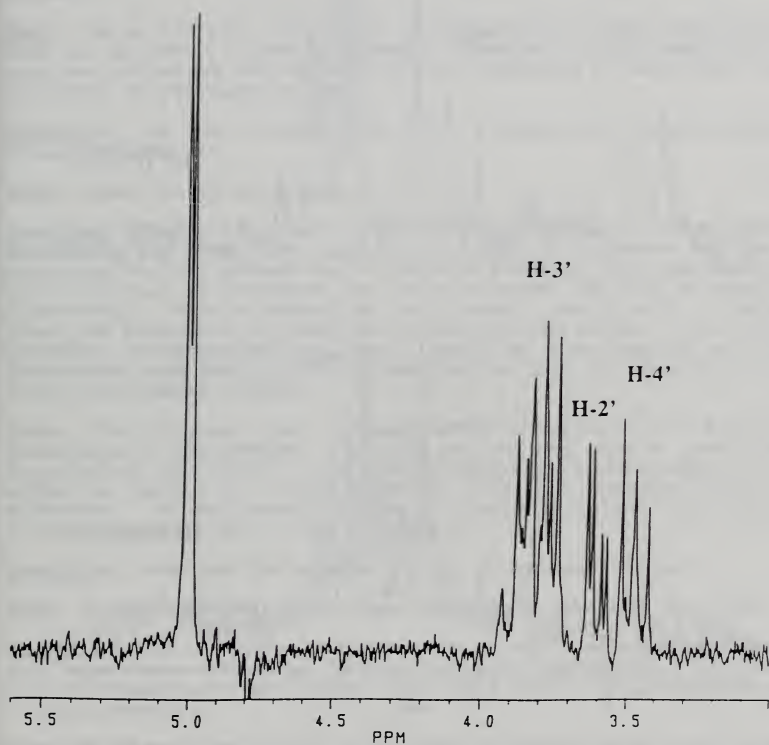


Figure 8. 200-MHz 1D TOCSY spectrum (τ_m 120 ms) of III-D, obtained by selective irradiation of Glc, H-1' at 5.00 ppm.

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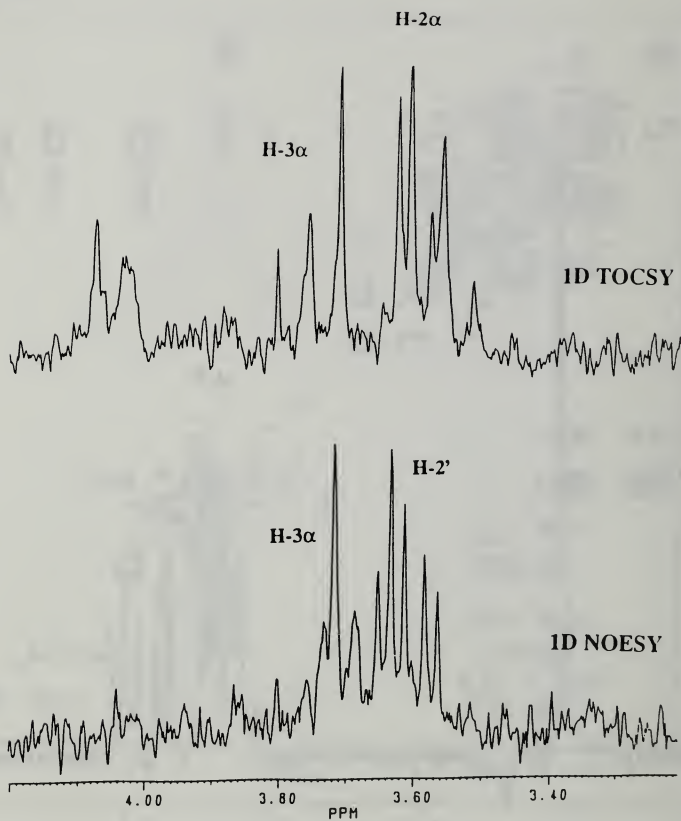


Figure 9. Expansion of the 1D TOCSY spectrum (τ_c 120 ms) of III-D, obtained by selective irradiation of Glc, H-1 α (5.29 ppm) and expansion of the 1D NOESY spectrum (τ_c 800 ms) of III-D, obtained by selective irradiation Glc, H-1' (5.45 ppm).

DISCUSSION

Question: Did you confirm the presence of glucose in your first hydrolysis using, for example, an enzymic method such as glucose oxidase?

Khan: No we did not. Of course there are many simple techniques that can be used to identify glucose. However, in this particular case our objective was to use the nmr technique to determine the structure of polysaccharides.

Question: In your sample, you have a blend of disaccharides, trisaccharides etc.

Khan: Yes, absolutely right.

Question: When you carried out the depolymerization to obtain more manageable nmr data, you used a dextranase. Is there any real advantage to that method over a hydrogen fluoride (HF) degradation which is now in standard use (for example, at the Arrhenius Laboratory). Using a commercial dextranase, I would worry a little about the formation of reversion products by the enzyme. Reversion products (oligosaccharides, etc.) could be more typical of the dextranase used than of the polysaccharide that you started with. Could you comment, please.

Khan: The original plan included both the chemical and enzymic hydrolysis of the dextran. We have not yet had time to carry out the chemical hydrolysis. The enzymic hydrolysis was preferred in order to obtain an oligosaccharide with its branched $\alpha(1\rightarrow3)$ structure. A dextranase, if pure, should hydrolyze the $\alpha(1\rightarrow6)$ linkages and not the $\alpha(1\rightarrow3)$ linkages.

Question: What was the source of your dextranase enzyme?

Khan: Sigma Chemicals - we found out that it was not very pure.

Question: Then I suggest that the major product is isomaltotriose, not isomaltose. There is, in fact, only one dextranase - and that is not commercially available - that will result in isomaltose as a major product.

Also, I am surprised that you show the branched trisaccharide and not some pentasaccharide or hexasaccharide. This enzyme was apparently able to get within one glycosidic linkages of the branch point, which is a bit unusual. Have you confirmed that this is a trisaccharide of the nmr spectrum?

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Khan: Yes, we are quite sure, because the fractions close to the total volume, as shown in the size exclusion chromatograph, contain mono-, di- and tri-saccharides. The trisaccharide is not pure. However, this fraction does not contain penta- or hexa-saccharides. The nmr also supported that in the trisaccharide (III-D), the isomaltose residue had an $\alpha(1\rightarrow3)$ branched glucose unit.

We also confirmed by the integration of both ^1H and ^{13}C spectra that the branching in the branched dextran was 70%.

Question: You have shown it as a trisaccharide but I believe it is more likely to be a hexasaccharide with extra $\alpha(1\rightarrow6)$ linkages. The enzyme would not have got that close to the $\alpha(1\rightarrow3)$ branch point.

Khan: That could be a possibility - I will not ignore it. We believe that the hexasaccharide is in the IIIC and IIIB fractions and not in the IIID. We have analyzed the IIID fraction.

Question: Also, thank you very much for showing how diverse dextrans are in nature.

Khan: Yes, they certainly are.

Question: You isolated the non-sugars simply by precipitation with methanol, and then characterized the polysaccharide fraction. Can you comment on what percentage of the non-sugars is of the polysaccharide type?

Khan: Yes, I showed you the figure where both refractive index and UV detectors were used and it was clear that colored compounds were associated with polysaccharides. No, we did not quantify the two components.

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RAW JUICE COOLING CRYSTALLIZATION: PILOT PLANT TRIALS ON CONTINUOUS
CONCENTRATION AND CRYSTALLIZATION STEPS.

Giuseppe Vaccari¹, Giorgio Mantovani¹, Giulio Squaldino¹ and Franco
Andreoli²

¹University of Ferrara, Ferrara, Italy

²Eridania Z.N. Spa, Genova, Italy

ABSTRACT

In the further scaling-up of the raw juice direct concentration and crystallization process, the authors moved from a discontinuous batch (Ref.: Int. Sugar J., 95, 1993, n. 1138, 381-390) to a continuous system by setting up two evaporation bodies and a continuous crystallizer.

The results obtained encourage the continuation of the experiments which would gradually allow the setting up of a counter-current evaporation battery and a crystallization system fitting the optimum cooling curves. These curves are based on laboratory growth kinetic data obtained in the laboratory.

INTRODUCTION

In previous papers (4-10) we have pointed out the reasons which led us to try to crystallize sucrose directly from raw juice, without any type of purification. Such reasons convinced some Japanese researchers to follow in the same way and they were able to obtain in their laboratory results which are quite similar to our own. In particular, Kawamoto and Sayama (3) conclude as follows: "1) the purity and recovery of the raw sugar by two stages of cooling crystallization were more than 98% and about 80%, respectively; 2) even though the highly coloured raw materials contain many impurities, by using the cooling crystallization procedure, we succeeded in obtaining raw sugar of good crystal shape and of uniform size; 3) while the raw sugar produced from conventional boiling crystallization contains many impurities, that produced from cooling crystallization had a transparent appearance with a clear shape; 4) as there were no purification steps, most of the non-sugars in the raw juice were concentrated into molasses; such a molasses was expected to be useful for the feed and fermentation industries".

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Owing to the particular characteristics of acidity and the thermolabile nature of raw juice, the concentration step must be carried out at the lowest possible temperature, and consequently at a high vacuum. Moreover, taking into account the peculiar colour and turbidity of the concentrated raw juice, it is absolutely necessary to crystallize via cooling. In fact, in this case, because we can maintain less drastic crystallization conditions, we can more completely utilize the "purification" power inherent in the crystallization process.

The scheme we have adopted, first in the laboratory and then in pilot plants of increasing size, is shown in Figure 1. The basic scheme of the discontinuous pilot plant, built by ERIDANIA Z.N. S.p.A. in the sugar factory of Finale Emilia, for the 1992 sugar campaign and described in a previous paper (8), is shown in Figure 2. By using such a plant, and with the addition of affination and filtration steps to the raw sugar produced, we were able to obtain white sugar of a commercial type as may be seen in Figure 3. The further steps which had to be adopted for the subsequent increase in plant size had necessarily to be related to a continuous concentration system with a greater number of evaporators, plus continuous cooling crystallization. The first steps towards this objective were also carried out in the Finale Emilia sugar factory during the 1993 sugar campaign.

EXPERIMENTAL METHODS AND RESULTS

The points which were taken into account during the 1993 experiments were as follows:

1. Continuous concentration of raw juice through two evaporator bodies heated in parallel, followed by an Alfa-Laval plate evaporator as a second body.
2. Investigation of the possibility of continuous counter-current concentration of raw juice.
3. Utilization of the only Alfa-Laval plate evaporator for the concentration of the run-off syrup used in the low boiling crystallization.
4. Utilization of a continuous vertical crystallizer for the first and second crystallization steps.
5. Evaluation of the analytical data obtained from products sampled during the various testing steps.

In the following we will discuss these various points.

1. Continuous concentration of raw juice using two evaporator bodies heated in parallel and an Alfa-Laval plate evaporator as a second evaporator body.

Following the progressive increase in the size of the concentration plant, the concentration system previously utilized, and made up by a single evaporator, was both significantly and basically modified to provide a battery of two bodies. In order to limit the experimentation costs, part of the previously installed facilities were utilized, in particular the forced-circulation evaporator. The latter, although having a high raw juice retention time, operated as a first body whereas an Alfa-Laval plate evaporator operated as a second body bearing in mind the reduced volume of liquid to be contained.

In the first series of experiments the two bodies were utilized according to the scheme shown in Figure 4. The heating of both bodies was obtained using the same vapour which came from the third effect of the sugar factory evaporator station. Continuous concentration ($0.6-0.7 \text{ m}^3$ of raw juice per hour) did not give any problem. In fact, no foam or scale formation was observed. As was already pointed out during the trials using only the forced circulation evaporator, (and also for the Alfa-Laval equipment), it was possible to avoid foam formation if the juice flow left the plates just above the liquid level inside the expansion vessel. Careful control carried out at the end of each trial confirmed that there were no incrustations or solid deposits capable of blocking or restricting the channels of the plate evaporator. Figure 5 shows one of plates after a long period of work. Bearing in mind that, compared with the working conditions normally encountered by the Alfa-Laval evaporators in traditional sugar processing, the temperatures in our experiments were relatively low, the gaskets were not damaged, although there were frequent inspections during the operations without having to change the gaskets themselves.

2. Possibility of continuous counter-current concentration of raw juice.

The change from concentration in parallel to counter-current concentration, such as is shown in the scheme of Figure 6, did not create any particular practical problem. Of course, it was necessary to share the work of concentration between the two bodies, so that the second body (Alfa-Laval) could provide all the vapour to heat the first body. Obviously, the number

of the plates of the Alfa-Laval evaporator was increased in order to comply with the concentration balance between the two bodies, and a higher output pump was installed for the recirculation of the juice to the second body. The system quickly became self-balanced because of the completely automated management of the plant.

3. Utilization of the Alfa-Laval plate evaporator as the only concentrator of the run-off syrup used in the low-boiling crystallization.

The run-off syrup from the first crystallization step (see below) was concentrated again in the Alfa-Laval evaporator, in order to carry out the second crystallization step according to the scheme shown in Figure 7. Also in this case there were no particular problems in spite of the increase in viscosity and the higher value of Brix chosen in comparison with the crystallization of first product sugar .

4. Utilization of a continuous vertical crystallizer for the first and second crystallization steps.

Again with the aim of gradually moving to possible industrial feasibility of the proposed working scheme, continuous crystallization must be installed after the continuous counter-current concentration step. As a first tentative stage, it was decided to re-utilize the continuous vertical crystallizer previously employed (4) for the cooling crystallization of thick juice. Such a crystallizer has been utilized for both first and second crystallization products according to the scheme shown in Figure 8. The concentrated syrup leaving the continuous evaporation plant was added with a suitable amount of seed before entering the continuous crystallizer. The seed was made from normally ground sugar suspended in saturated pure glycerol. The magma leaving the crystallizer continuously at about 40 °C, entered an intermediate stirred tank where the temperature could be further decreased. The system gave good practical results (as had also been found for the thick juice crystallization), although it has to be borne in mind that for further increase in throughput this crystallizer must be significantly modified. In fact, we have to take into account that, even though the crystallizer was suitably insulated, the magma cooled abnormally and not following a programme via the control of the cooling water temperature. This fact, together with the irregular flow entering of the syrup, interfered with the optimum conditions of seeding and favoured spontaneous nucleation phenomena during cooling. These latter were also promoted because it was impossible to programme different

slopes of the magma cooling curve during the whole crystallization range. However, it was possible to verify that the solubility conditions determined by previous laboratory trials (11) were sufficiently reliable during the whole period of the sugar campaign. In spite of these problems and the difficulties due to the non-industrial size of the centrifugal which we had to employ, we were able to obtain crystallized first and second product sugars whose qualities were comparable with those obtained in the previous campaigns using discontinuous crystallization, as is shown in Table 1. Figures 9 and 10 show crystals of first and second products.

No particular technical difficulties were encountered when going from first to second product crystallization. Problems related to occasional foaming of magma, which were observed in the previous trials during the second product batch crystallization, were never observed during continuous crystallization.

Figure 11 shows a view of the pilot plant as a whole.

5. Evaluation of the analytical data obtained from products sampled during the various testing steps.

During the various testing steps, suitable samples were collected from the various points in the plant, in order to follow the various process steps from the analytical point of view. We have to bear in mind that, although the whole plant was automated via a computer, the operational conditions of the continuous plant are still far from optimum, and, in particular, concerning the juice retention time inside the first and second concentration bodies. Taking into account that the volume of the forced-concentration evaporator, acting as first body, was very oversized in respect to the operational characteristics of a system with programmed flows, the juice retention time was about 4 hours. That is really too long because of the possibility of chemical and microbiological transformation of sucrose.

In the second body (Alfa-Laval), the retention time was one hour but this was still too high if we take into consideration the relatively high temperature in the final concentration step.

The choice of these operational conditions was advised both by the necessity of using, at least partially, the previous plant, as well as presenting an opportunity to test difficult operational conditions so as to try to control the relevant effects. A marked decrease in the retention time, scheduled for next campaign, might considerably decrease the sucrose

inversion and destruction effects. However, these were not particularly serious even under these operational conditions.

As far as sucrose inversion is concerned, it is known that the invert content in the juice promotes a decrease in the sucrose solubility (2, 13). However, invert is formed at the expense of sucrose, and the question is what is the optimum level of invert to be aimed for? The answer to this question is beyond the scope of this paper, but it can be obtained via a suitable balance, which takes into account the effect exerted by invert on sucrose solubility.

In Figure 12 the average data for concentration of invert % juice and invert % sucrose in the various process steps are depicted. However, the increase of the invert content does not represent as such all the sucrose which has been destroyed. This latter amount of sucrose can be calculated through a careful balance, which can be made on the basis of sugar entering and leaving the various process steps. Figure 13 shows the amount of sucrose (expressed as grams/100 grams sucrose), which was inverted and destroyed, respectively, during the various stages of the process. We can see that, as mentioned above, although the worst conditions related to the long retention time during concentration, the amount of sucrose destroyed was relatively small. Sucrose destruction, which first starts via the disaccharide inversion and then through the further transformation of monosaccharides, involves glucose even more than fructose. This is quite clear from the data shown in Figure 14 where the variation of the glucose/fructose ratio in the various process steps is reported. Of course other non-sugar components can undergo chemical modification during the various process steps. An example is glutamine which forms pyrrolidone carboxylic acid (PCA) by losing ammonia (1), as shown in Figure 15.

CONCLUSIONS

We believe that the results which we have obtained can be considered to be a further step towards the industrial and productive realization of the technology of direct concentration and cooling crystallization of raw juice. A further increase in plant size might provide the installation of a counter-current battery made up of a number of Alfa-Laval plate evaporators, having minimum juice retention time in the various bodies. As far as crystallization is concerned, the experience gained in this first year of trials with the continuous crystallizer previously employed some years ago for thick juice crystallization, has pointed up some problems. These concern continuous crystallization of solutions having high Brix (for increasing the crystal sugar yield), if we want to decrease

the sugar inversion and destruction phenomena as much as possible. We believe that such a problem could be solved by dividing the cooling crystallization stage into a number of steps, with the first utilizing an adiabatic crystallization. This solution would not only avoid starting the cooling crystallization at too high a Brix and high temperatures, but would also favour the initial steps of crystallization and limit the problem of spontaneous nucleation. The later crystallization stages might be achieved optimally by adopting cooling rates calculated from the knowledge of the solubility data (11) and of kinetic growth figures which we recently presented to the meeting of the C.I.T.S. Scientific Committee in Warsaw (12). Of course, special attention will have to be given to the conditions of sterility in the diffusers so as to avoid the possible formation of volatile acids (acetic, propionic or butyric acids) during the extraction which can pollute the condensate. In fact there is not a high probability of microbiological activity during the evaporation, bearing in mind that the retention time must be very short, while the flash evaporation conditions and the high sucrose concentration will inhibit microbiological proliferation. On the other hand, volatile acids in the condensate could become a problem both for their recycling and disposal. Furthermore, careful bacteriological examination will be carried out on the refined sugar produced. Additionally, the fermentability characteristics of the molasses will be considered in view of the possibilities of utilization in biotechnology.

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Table 1. Characteristics of non-affinated 1st and 2nd product raw sugars

1st raw sugar - not affinated		
	1992	1993
Purity	98.2	98.4
Invert %	0.32	0.25
Ash %	0.45	0.35
Colour In solution (I.U.)	1600	940
2nd raw sugar - not affinated		
	1992	1993
Purity	97.6	98.2
Invert %	0.35	0.24
Ash %	0.48	0.23
Colour In solution (I.U.)	2500	2200

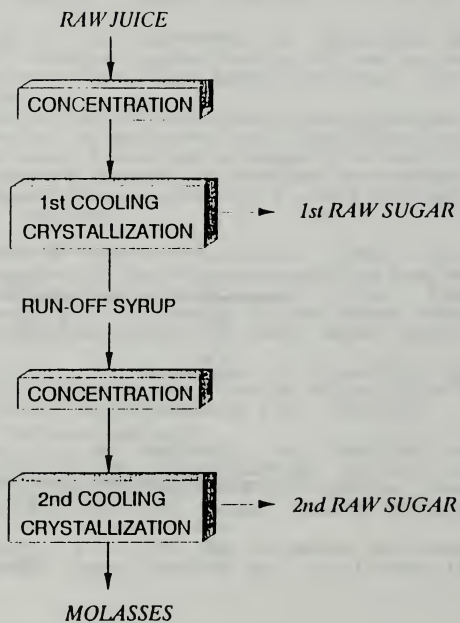


Figure 1. Flow diagram of the two-stage raw juice crystallization scheme.

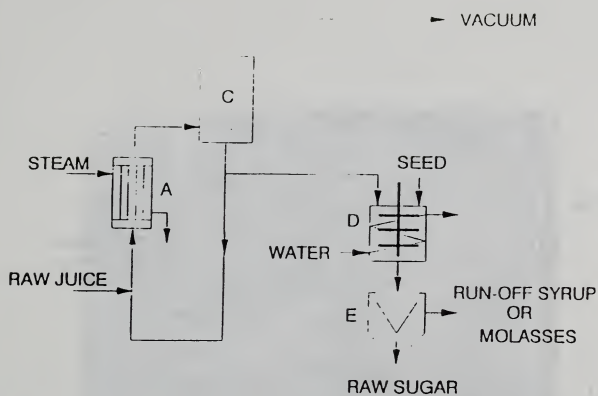


Figure 2. Discontinuous pilot plant scheme: A) heater; C) flash evaporation, D) crystallizer, E) continuous centrifugal.

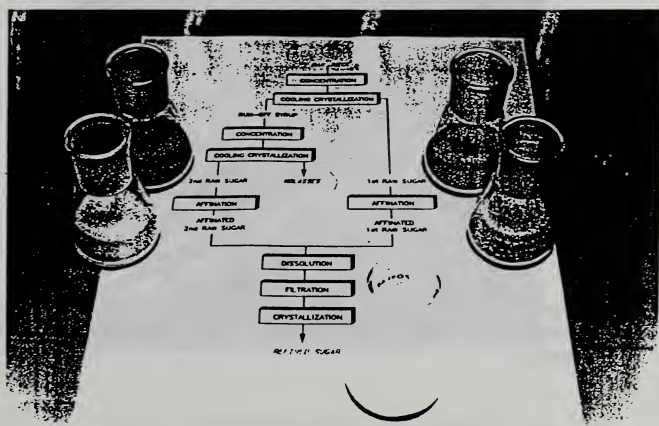


Figure 3. Flow sheet for the direct production of refined sugar from raw juice

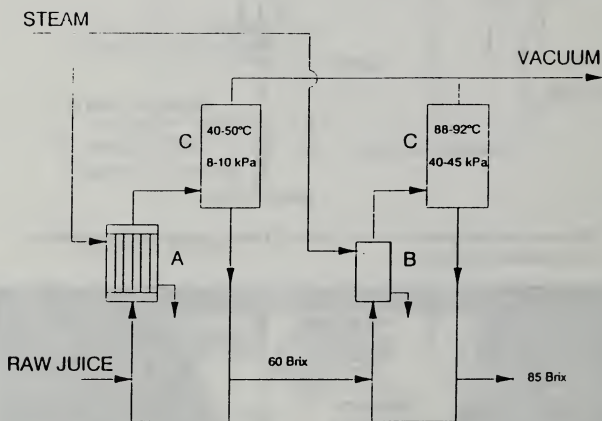


Figure 4. Scheme of continuous concentration using two evaporator bodies heated in parallel: A) forced-circulation evaporator; B) Alfa-Laval heater; C) expansion vessels.

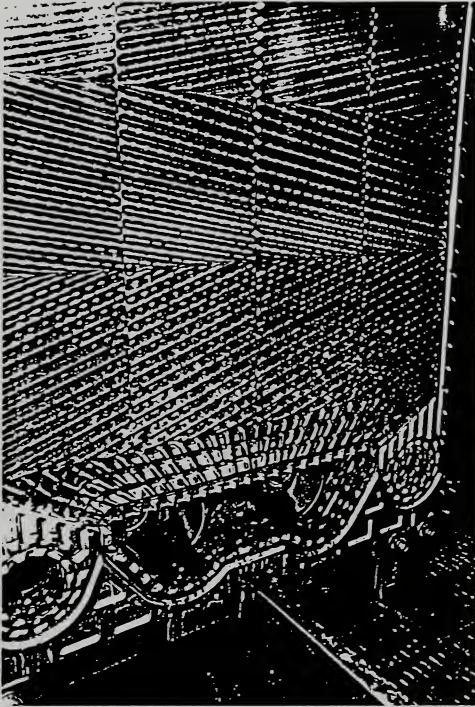


Figure 5. Detail of the Alfa-Laval heater plates.

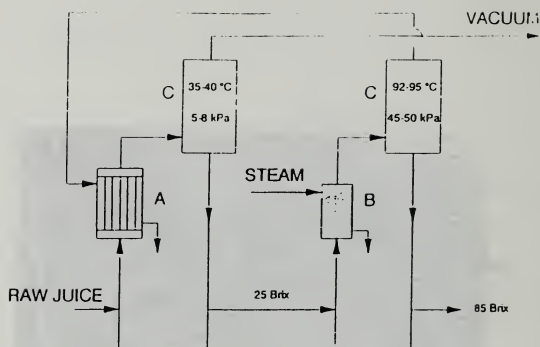


Figure 6. Scheme of continuous concentration using two evaporator bodies heated counter-currently: A) forced-circulation evaporator; B) Alfa-Laval heater, C) expansions vessels.

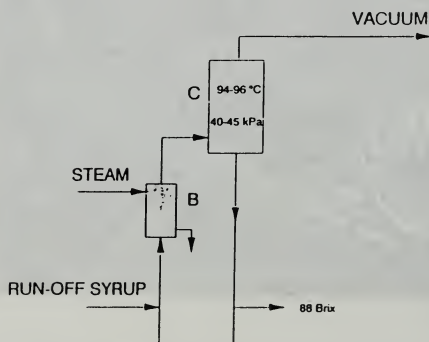


Figure 7. Scheme of the run-off syrup concentration: B) Alfa-Laval heater, C) expansion vessel.

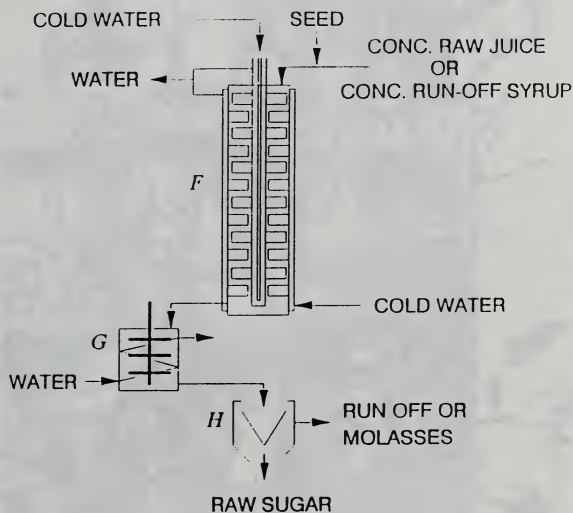


Figure 8. Scheme of continuous crystallization: F) continuous vertical crystallizer; G) magma tank; H) continuous centrifugal.



Figure 9 First product magma crystals

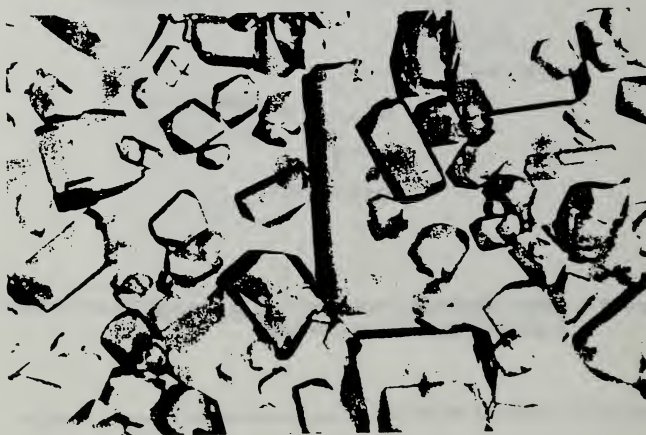


Figure 10 Second product magma crystals

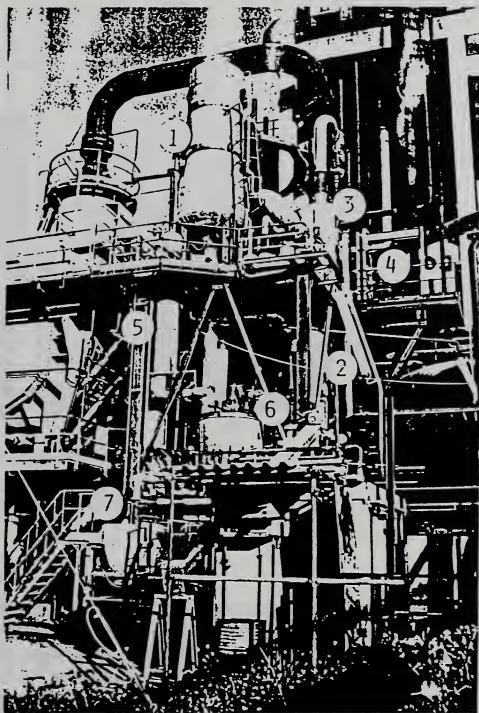


Figure 11. Pilot plant for continuous concentration and crystallization of raw juice: 1) 1st flashing; 2) 1st heater; 3) 2nd flashing; 4) 2nd heater (Alfa-Laval); 5) continuous cooling crystallizer; 6) mixer tank; 7) continuous centrifugal

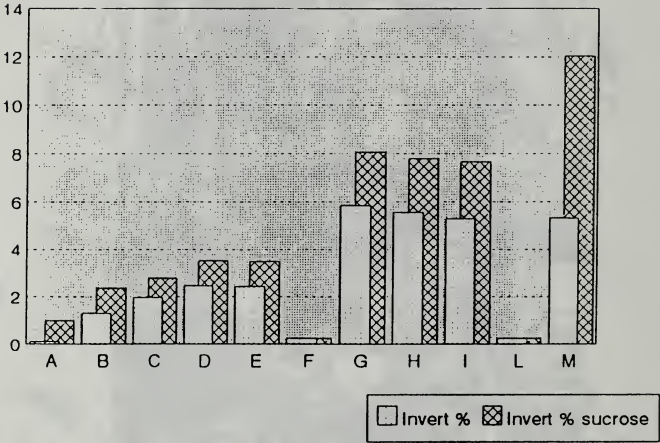


Figure 12. Invert content in the various process steps: A) raw juice; B) 1st concentration loop; 2) 2nd concentration loop; D) 1st product magma leaving the crystallizer; E) magma leaving the mixer tank; F) 1st product raw sugar; G) run-off syrup; H) concentrated run-off syrup; I) 2nd product magma; L) 2nd product raw sugar; M) molasses.

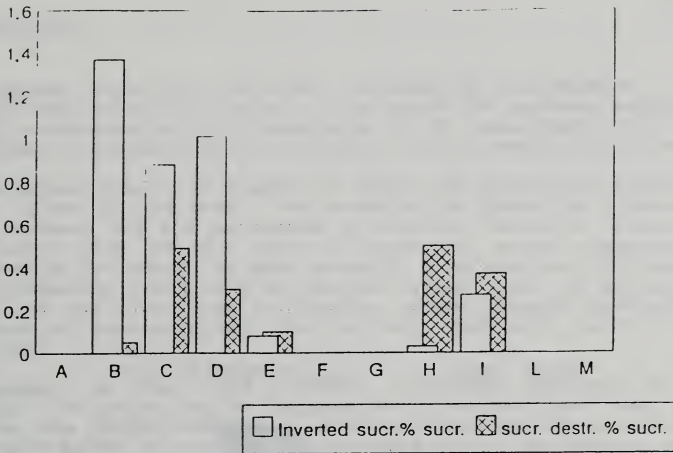


Figure 13. Inverted sucrose or sucrose destroyed in the various process steps (For the letters key, see Figure 11).

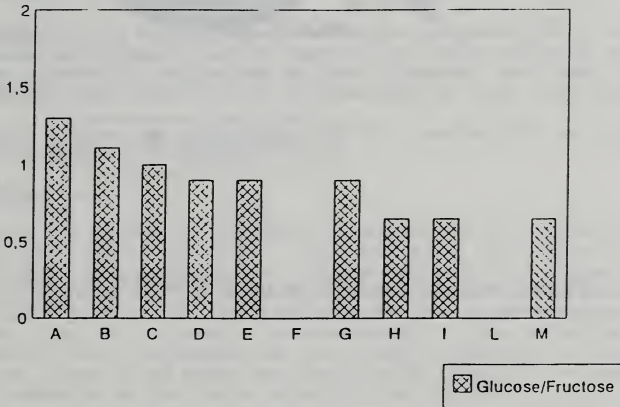


Figure 14. Variation of the glucose/fructose ratio during the various process steps (For the letters key, see Figure 11).

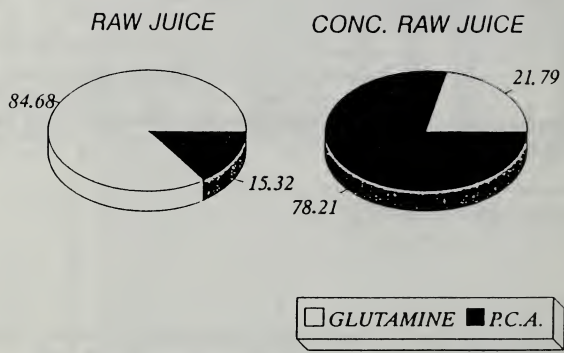


Figure 15. Glutamine transformation to pyrrolidone carboxylic acid during raw juice concentration.

DISCUSSION

Question: Thank you for a very interesting presentation. I am a great admirer of this project - you blend the academic science and deep understanding first developed in the laboratory with a very pragmatic approach for the factories. You are making good progress.

A question related to Figure 13, about the percentage destruction of sucrose in each step - the formation of invert as a percentage of sucrose, and the percentage of sucrose destroyed: what do you consider will be the optimum amount of sugar destroyed, or converted to invert, in this process? When you have finished your developments - next year or the year after - what do you consider will be the total loss of sucrose, either through conversion to invert or through direct destruction? What will be the optimum figure?

Vaccari: We would like to avoid completely the destruction of sugar. We are trying to decrease the retention time. But, we can allow the partial inversion of sucrose and we can calculate the optimum amount of sucrose that we can invert. Taking into account the decrease in solubility we can obtain by increasing the invert content and decreasing the sucrose content, we can find a balance using the solubility curve we obtained two years ago in the laboratory.

Question: If you did not consider further refining the product from your cooling crystallization, what do you think would be the nature of the non-sugar inclusions in your crystals, and what impact do you think they would have on further use of that sugar product?

Vaccari: You are referring to the possibility of using the crystals without refining?

Question: Yes.

Vaccari: We obtain two kinds of products, after affination of the first and second product. As you see, the color decreases drastically after affination, because the amount of impurity inside crystal made by cooling crystallization is very low. The kinds of compounds present in the crystal are the same as those in the juices. In our opinion, the non-sugars in the crystals are the result of the entrapment of very small droplets of raw juice. However, even if we adopt cooling crystallization, we can't avoid small amounts of raw juice entering the crystal.

Question: In your personal opinion, do you believe that the presence of those non-sugars would be an obstacle to the use of that sugar product without further refining?

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Question: In your personal opinion, do you believe that the presence of those non-sugars would be an obstacle to the use of that sugar product without further refining?

Vaccari: In my opinion, no.

Question: Do you believe that cooling crystallization is inherently better - gives better crystals, with less color transfer - than evaporative crystallization? Have you run a controlled experiment, carrying out evaporative crystallization very slowly, to match the rate of cooling crystallization.

Vaccari: The main reason we can obtain good quality crystals from cooling crystallization is that we can crystallize slowly. If it were possible to crystallize slowly in evaporation, the magma would have to be maintained at high temperature for a long time, causing destruction of sucrose and other components. In traditional technology, we try to decrease the time in the boiling pan. In my opinion, it is not possible, from both economic viewpoints and the quality of the sugar, to maintain magma at high temperature for a long time.

There is another point: crystallization by boiling disturbs the regular growth of the crystal because formation of bubbles of vapor around the surface of the crystal changes the interface conditions between crystal and solution, and changes the saturation- it is more difficult to control crystallization conditions in heating than in cooling.

Question: To go back to the point about temperature: in order to get a sufficient yield, you have to start at 85 Brix; so, you have to go to a much higher temperature to start than you would have in evaporative crystallization. So although the average is a lower temperature, the initial temperature is higher in cooling crystallization.

Vaccari: Yes, if we want to increase the yield of sucrose crystallized, we need to increase the Brix and temperature. We think that, in future, it would be best to stop concentration at 85 Bx and 88°C-91°C. We can gain another 2 or 3 Bx if we start the cooling crystallization by adopting adiabatic crystallization. When we reach a fixed Brix, we add seed under vacuum, then evaporate slowly without heating to remove some water to increase Brix during the first stage of crystallization. In this way we can increase the yield, without using very high temperatures.

SUGAR FACTORY INSTRUMENTATION AND AUTOMATION STRATEGY

Sven Barfoed and Visti Andersson

Niro Sugar & Sweetener Plants Division, Søborg, Denmark

ABSTRACT

All over the world sugar factories are being modernized and automated in response to the need for efficient production in a competitive market. This paper describes the automation strategy recommended by Niro Sugar & Sweetener Plants Division for factories ranging from manually to semi-automatically operated. Emphasis is on the requirement of reliable and dependable operation under all circumstances. The strategy is illustrated by the optional installation alternatives of our newly developed vacuum pan processor.

INTRODUCTION

In connection with the automation of a factory it is important to establish a well defined strategy for this process - stating the current stage, the final goal, and the way to reach the goal.

The strategy should be as detailed as possible and go so far as to describe the operating concept for motors, valves, the principles for alarm handling, etc. This "Manual" can be used in connection with the reconstruction of existing plants and the purchase of new equipment. Thus a uniform structure and operation of the entire factory will be achieved

Today much equipment is automated using relay based systems, PLC-systems or other types of electronic control (1). If there are no firm guidelines in this respect the result may be a mixture of different types of control equipment, with regard to both the operation concept and the appearance, to the irritation of the operators and the maintenance personnel.

Furthermore, lack of strategy can make it be impossible to upgrade from one automation level to another without having to change a lot of the equipment.

A strategy for the modernization of a factory will be described, where the emphasis will be on the selection of hardware- and software systems together, with examples of these.

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CHOICE OF STRATEGY

The automation systems can roughly be divided into two main groups:

- Centralized Computer System (CCS).
A system based on a powerful computer that controls and collects data for the entire factory.
- Distributed Computer System (DCS).
The factory is divided into small process sections each with a process computer attached.

To change from manual control, with control panels placed locally, to a fully automatic Centralized Computer System would be a very ambitious and cost-consuming project. Instead it is recommended to install a Distributed Control System (DCS), which is then gradually extended process section by process section. This results in a simple and reliable system, where the different modules can function as standalone units - independent of the operating conditions for the others modules.

In the DCS all process units can be interconnected via a computer network to a supervisory system, which consists of one or more main control computers. However it is possible to implement the supervisory system at a later stage, if financial or other reasons should require so.

If these ideas concerning a decentralized modular design are continued to the supervisory system itself, you will end up with a personal computer platform consisting of standard modules for both hardware and software.

As shown in Figure 1: Level 0 basics level 1, level 1 basics level 2 etc. It is the policy of Niro to make it possible to stop the automation process at any level, and eventually continue upgrading at a later state. It is also policy to design the automation systems so that upgrading from one step to the next can happen with a maximum reuse of equipment.

A further advantage of a DCS is that, because it consists of a number of smaller units, eventual electronic failures will have only a limited effect. If required, a high degree of redundancy on critical parts can be implemented for a reasonable cost.

DECENTRALIZED COMPUTER SYSTEM

The vacuum pan in a sugar factory constitutes a unit with very well-defined borders to its surroundings; it is therefore especially suitable for automation according to above principle.

Niro vacuum pan processor

On the basis of the newest technology within automation systems Niro has developed an integrated control system for a vacuum pan - the Niro Vacuum Pan Processor.

The Niro Vacuum Pan Processor consists of a PLC-system, preprogrammed with the latest know-how of the crystallization process. This makes it possible to improve the process considerably over a plant with manual control, thus achieving a better product at lower energy consumption.

The following are characteristics of the integrated automation system:

- The equipment is sturdy enough to be locally mounted in immediate vicinity of a vacuum pan.
- Equipment can function separately or linked up with other vacuum pan processors and is prepared for connection to a supervisory system.
- Equipment consists of standard components, which are easy to exchange in case of an error.
- Equipment has all control loops for optimum control of the boiling process. Fully automatic control of all valves and stirrer with surveillance of the attached components.
- Integrated printer with alarm lists and curves.
- Manual control of all functions, which enables the operator to intervene no matter where in the sequence.
- Man/Machine Interface consisting of a 10" LCD-display with integrated light. The process is displayed graphically, which gives the operator a good survey. The control takes place through function keys placed under the display.
- Divided into function blocks - LCD-display, printer, controller outputs. These can be connected according to individual requirements.

The Niro Vacuum Pan Processor is a flexible system suitable for the modernization of older plants, while enabling improvements of the level of automation.

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Niro evaporation processor

The evaporation section constitutes a suitable section for automation. An optimum evaporation requires:

- to keep a constant high Brix of thick juice or syrup
- to minimize the energy consumption for evaporating, and
- to make the work routines more efficient.

Depending on the specific plant, the control will consist of a number of interdependent control loops, various control functions, and a general alarm supervision, which are to be integrated into a unit.

Niro has pursued the concept of the Vacuum Pan Processor and developed a similar control for the evaporation section - the Niro Evaporation Processor. It consists of the same components and has the same design and layout as well as a similar operating principle.

A Niro Evaporation Processor, as shown in Figure 2, is presently being projected for a factory in Mexico and is to be ready for the crop start in the autumn.

Other sections for which systems are available are: juice purification, extraction, and the sugar boiling house.

SUPERVISORY SYSTEM.

The supervisory system consists of a graphic user interface with interface to DCS, from where it is possible to control, supervise, and collect data from the individual processes in the factory. The information "goes" from the floor of the factory to the control room and the offices. Here these data can form the basis of a follow-up on production, increasing the efficiency and maintenance planning.

The objective of the supervisory system is to make the various production equipment from different suppliers form an integrated whole, where everything runs in real time, i.e. that "real" data are collected and displayed. In this connection it is important that all process sections are integrated, including the old control - and instrument systems.

Subsequently the supervisory system can be integrated into the other EDP-systems of the factory, so it will be possible to exchange data, for example, for production planning and statistical purposes.

Based on the above the following main criteria for equipment specification can be established:

The supervisory system software has to function on different data platforms, with OS/2 and Windows as the primary. It must be able to use the data world's open standards, so, in real time, data can be exchanged with other PC program packages e.g. spreadsheets. This gives access to the very large range of existing software for PC-systems. The software must include I/O drivers for as large a selection of different control systems as possible and it has to be able to handle several of these simultaneously.

There have been several definitions for a standard protocol for data communication for automation systems, but none of these has really succeeded and the market today contains a large number of different types of protocols. The software must be simple to configure both regarding the structure of the database and the graphic display. Likewise the operating interface has to be built up in a logical and userfriendly way and contain the facilities you might expect from a modern supervisory system - such as alarm system, trend system, report generator, high resolution graphics, etc.

From many possibilities, FIX DMACS, which is a PC-based program package for industrial automation, developed by Intellution Inc. USA, was chosen.

Figure 3 shows FIX DMACS integrated with a number of vacuum pans and with process automatics of a different make. These products cannot communicate directly, but can be integrated into a unity through the supervisory system.

The Niro Vacuum Pan Processor, Niro Evaporation Processor and others are developed for connection to a superior operating level or for standalone operation with the possibility of parallel operation of all functions.

Several PCs are integrated into a network, which gives the possibility of more workstations which can be placed anywhere in the plant, thus achieving higher operating safety, as an error on one of the operating stations only influences that specific station, while the rest still can be used.

SUMMARY AND CONCLUSIONS

The strategy outlined herein yields a flexible and reliable system, which can be gradually extended both in size and functionality according to customers' demand.

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Automation systems are developing quickly towards new and faster data communication systems, and in general, moving towards open decentralized systems. A system built according to this principle gives the highest security for the future and the widest choice among the best equipment available, as no single make is specified.

It is also possible to choose equipment dedicated to special process sections produced by process experts and thus achieving process know-how, as for example, with Niro's vacuum pan.

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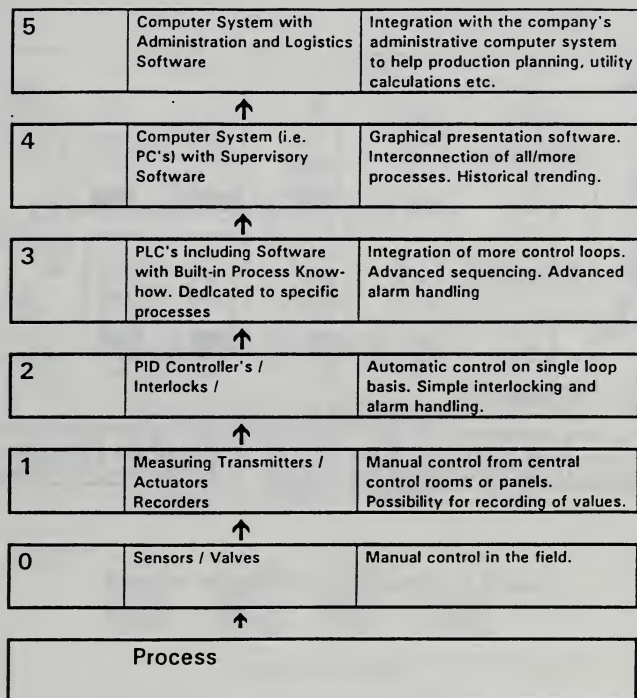


Figure 1. Development of levels of automation.

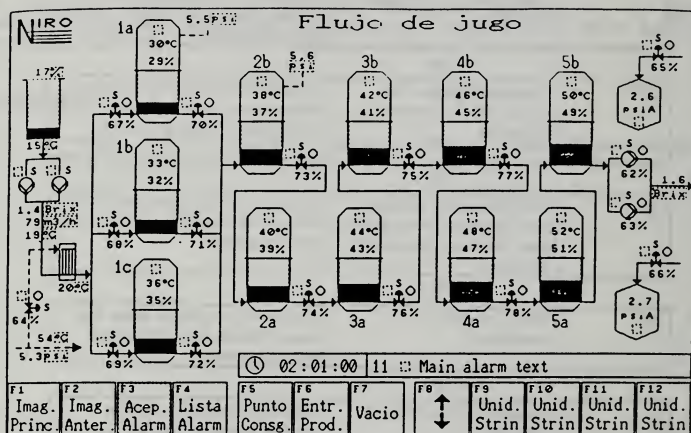


Figure 2. Typical evaporation process.

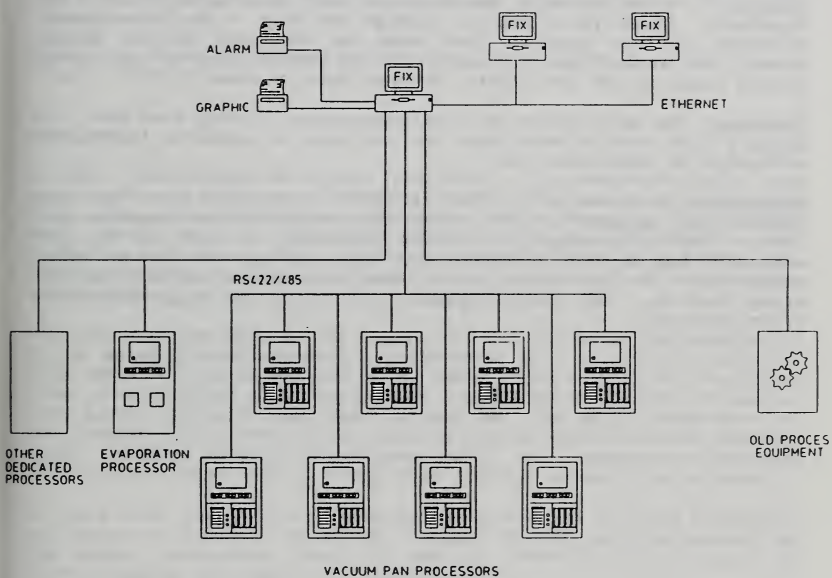


Figure 3.

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DISCUSSION

Question: In the abstract of your paper, you describe the vacuum pan processor, the DP6, as a new processor, but I did not understand exactly what is new - the configuration of the hardware or a new operating principle?

Andersson: It is based on a newer technology than the former designs. A brick work design (modular) is chosen to put it together. There is no special design for that - it is equipment selected and put together from what is available on the market today. The other type, previously on the market, was of the analog system type, or of one digital system made by hand.

Question: The development of instrumentation is very fast now. Is there a risk that in some years you may have an obsolete instrument connected to something new?

Andersson: In a few years, that is always a possibility. When you choose your equipment, you really have to go to the producer and look at his philosophy in making products, to see if the new equipment can be used together with older designs of that or other producers. You must also look at the development state - how far along that is. But new equipment must always be compatible with older systems.

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Helsinki, Finland, August, 1994

EFFECT OF MANGANESE AND VERATRYL ALCOHOL ON THE COLOUR REMOVAL FROM
SUGAR REFINERY EFFLUENTS BY *PHANEROCHAETE CHRYSOSPORIUM*

Carla B. C. Guimarães¹, Luís S. M. Bento¹ and Manuel G. Mota²

¹RAR - Refinarias de Açúcar Reunidas SA, Apt. 1067, 4101 Porto
Codex, PORTUGAL

²Dep. Eng. Biológica, Univ. of Minho, Largo do Paço, 4719 Braga
Codex, PORTUGAL

ABSTRACT

At RAR - Refinarias de Açúcar Reunidas - ion exchange resins are used to remove colourants present in sugar liquor after carbonation. The pre-regeneration of these resins is made with 50 g/l NaCl, giving rise to a heavily coloured effluent exhibiting a high phenolics content. The compounds present in this effluent are poorly degraded by the organisms normally involved in waste water treatments.

Previous studies made in our laboratory demonstrated the possibility of using *Phanerochaete chrysosporium* to treat this effluent. This organism has an enzymatic system related with lignin degradation, and is also able to degrade xenobiotics present in wastes from several chemical and food industries. The major components of the ligninolytic system are two families of extracellular peroxidases, namely lignin peroxidase (LiP) and manganese-dependent lignin peroxidase (MnP), along with the H₂O₂ generation system.

In this work the effect of manganese, essential for MnP activity, and veratryl alcohol, a normal inducer of LiP, on the removal of the sugar colourants from the resins regeneration effluent by *Phanerochaete chrysosporium*, was studied.

It was observed that the presence of veratryl alcohol (2 mM) did not improve the decolourization. To test the influence of manganese, concentrations of 0; 30; 60; 120; 165 and 727 µM of added Mn(II) were assayed. The concentration of 60 µM of Mn(II) was shown to be the optimal concentration for this cation, as far as colour and phenolics removal is concerned - about 63% of colour and 73% of phenolics removal was achieved.

INTRODUCTION

Ion exchange resins have proved to be excellent sugar liquor decolourizers. Effluents resulting from salt regeneration are a disadvantage of this process as they represent an environmental problem. Different processes have been proposed to overcome this situation. One process involves anionic colourants precipitation with lime (2). The colourants not removed by this process result from the pre-regeneration stage at low salt concentration (50 g/l). This fraction of effluent still represents an environmental problem due to the presence of phenolic compounds, intense colouration and high organic load (COD). The high toxicity of phenolic compounds to living organisms is well reported in literature (6,13). The brown colour of the effluent is not only aesthetically unacceptable but also inhibits the natural process of photosynthesis in natural waters leading to a chain of adverse effects on the aquatic ecosystem as the growth of primary consumers as well as secondary and tertiary consumers is affected.

The presence of phenolic compounds and caramels in the effluent impairs the traditional biological waste water treatment. Several authors reported that phenolic compounds (3,16,26) and caramels, such as furfurals (1), are inhibitory to methanogenic bacteria involved in anaerobic waste treatments. On the other hand, several phenolics are well known as disinfectants for many microorganisms and inhibit, at least partially, aerobic treatment processes (18). Another problem is the fact that most microorganisms normally involved in biological treatments are not able to degrade a broad spectrum of structurally diverse compounds, as it occur in the pre-regeneration effluent, thus restricting their use to situations where only a limited number of pollutants are present, or forcing the use of sometimes hard-to-maintain consortia of microorganisms, each of which possess the requisite enzymes to degrade one or, at best, a few compounds (8).

The white-rot fungus *P. chrysosporium* is a potentially useful microorganism in waste treatment systems because it is able to degrade a broad spectrum of structurally diverse organic compounds (9), such as DDT, lindane, ferulic acid, benzo(a)pyrene, chlorinated aromatics, etc (8). Indeed, previous studies made in our laboratory demonstrated the possibility of using *Phanerochaete chrysosporium* to treat the effluent from the pre-regeneration of decolourizer resins (28).

Evidence suggests that the unique ability of *P. chrysosporium* to degrade those persistent compounds is due, at least in part, to the lignin degrading enzymatic system of this microorganism (8,9), that is non-specific and partially extracellular.

The major components of the ligninolytic system are two families of peroxidases, namely lignin peroxidase (LiP) (30) and manganese-dependent lignin peroxidase (MnP) (24), along with the H_2O_2 generation system (22). LiP's catalyse H_2O_2 -dependent one-electron oxidation of a variety of lignin-related aromatic compounds resulting in the formation of unstable aryl cation radicals. These radicals undergo various non-enzymatic reactions yielding a multiplicity of end products (5,22,29). The reactions involve carbon-carbon bond cleavage, aromatic ring opening, demethoxylation, hydroxylation, decarboxylation and phenol coupling reactions (5,22,29). MnP's catalyse the H_2O_2 -dependent oxidation of Mn(II) to Mn(III) as well as some phenolic substances. Mn(III) can then oxidise other compounds (10). These enzymes could not efficiently complete the catalytic cycle in the absence of Mn(II), suggesting the absolute requirement of Mn(II) (31). In the degradation of lignin, Mn(III) can oxidise phenolic lignin substructures, leading to phenoxy radical intermediates (29). Subsequently, C α -C β cleavage or alkyl-phenyl cleavage would yield the depolymerised fragments, including quinones and hydroquinones.

Several studies made with synthetic culture media demonstrated that lignin peroxidase (LiP) activity of *P. chrysosporium* is stimulated by incubating cultures with veratryl (3,4 - dimethoxybenzyl) alcohol, a secondary metabolite of this fungus (11,14,15,23,25). The increase in lignin peroxidase activity can be attributed to the ability of veratryl alcohol to protect lignin peroxidase from H_2O_2 inactivation (11,19,20,21) and to function as low molecular weight electron mediator between substrate and peroxidase (19-22). It was also demonstrated that the addition of veratryl alcohol to the cultures did not alter the Mn peroxidase activity (11).

Recent studies showed that LiP or MnP production can be selectively affected by regulating manganese levels in the culture medium of *P. chrysosporium* in presence of veratryl alcohol (4,7,27). LiP titres varied as an inverse function of and MnP titres varied as a direct function of the Mn(II) concentration. Addition of 727 μ M of manganese to the medium resulted in no LiP production and a great enhancement in MnP production.

In this work we intended to investigate the effect of veratryl alcohol and manganese on the removal of sugar colourants from the effluent by *P. chrysosporium*.

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METHODS AND MATERIALS

Organism and inoculum

Phanerochaete chrysosporium (ATCC-24725) was kindly supplied by Prof. J. Lema (Univ. Santiago Compostela, Spain) and was maintained on agar medium containing 1% glucose, 1% malt extract, 0.2% peptone, 1% yeast extract, 0.1% asparagine, 0.2% KH_2PO_4 and 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. This medium was also used for spore production. Inoculum consisted of filtered (glass wool) conidial suspension in sterile water ($A_{450\text{nm}} = 0.500/\text{cm}$, approximately 2.5×10^6 spores/ml).

Effluent

The effluent was collected in the Refinery during the regeneration of the decolourizing resins with 50 g/l NaCl. The final salt concentration of the collected effluent was 20 g/l. As it had been previously shown (28) that this concentration reduces the fungal growth rate, the effluent was diluted to a final salt content of 15g/l.

Biological treatment

Effluent at pH 4.5 was supplemented with Na acetate buffer at pH 4.5, basal medium, glucose (0.2%) and thiamine (1 mg/l). The basal medium was composed of several minerals as described in (28). Effluent, basal medium, glucose and thiamine were filter sterilised. Na acetate buffer was autoclaved (121°C, 15 min). Nitrogen was not added because the effluent contained enough nitrogen (4-6 mM) to sustain growth. The supplemented effluent (50 ml) was dispensed into sterile cotton-stoppered 500 ml flasks and inoculated with 5 ml of the above mentioned conidial suspension. This inoculum was replaced with sterile water in the control flask (inoculated control). The organism was grown in shallow stationary cultures at 39 °C, during 26 days.

Mn(II) was added at the time of inoculation, as part of the basal medium. The concentration of added manganese was as follows : 0 μM , 30 μM , 60 μM , 120 μM , 165 μM and 727 μM .

Veratryl alcohol (2 mM) was added at the time of inoculation. Control cultures received an equivalent volume of water.

Each assay was done in triplicate. Standard deviations were always less than 10%.

Colour

Colour, expressed as attenuation*, was measured at 420 nm, pH 9, with a Perkin-Elmer LCC-55B spectrophotometer.

$$\text{*Attenuation} = (\text{Absorbance} \times 1000) / \text{cell length, cm}$$

Phenolic compounds

Phenolics were determined as "phenol-reacting substances" using the Polin and Ciocalteu reagent (12,17).

Gel filtration chromatography

A Pharmacia FPLC system was used, equipped with a Superose-12 column. The detector was a L-4500 Merck-Hitachi Diode Array and the software was model D-6500 Merck-Hitachi. 200 μ l of sample (filtered with 0.45 μ m Gelman filter) was eluted with acetonitrile 30% (v/v) + NaAcetate 0.1 M, pH 8.0, at a flow rate of 0.50 ml/min.

RESULTS AND DISCUSSION

Mn(II) concentration is considered as the concentration of manganese added to the cultures, not to the total Mn(II) concentration, since effluent has always manganese traces (3-4 μ M).

Effect of manganese in the absence of veratryl alcohol

All previously reported experiments about the effect of manganese in LiP and MnP were made using veratryl alcohol. A study on the effect of manganese without veratryl alcohol would however be interesting, and that was one of the aims of this work.

Various concentrations of Mn(II) were added to the effluent prior to treatment by *P. chrysosporium*. To evaluate the fungus performance the colour and phenolic compounds were measured after fungal treatment.

The reduction of colour and phenolic compounds, obtained after treatment, increased with increasing Mn (II) and reached a maximum at 60 μ M (Figure 1). Above 60 μ M the removal was maintained at a constant level, even when the concentration of Mn (II) was increased to 727 μ M (Figure 2).

Gel filtration chromatography confirmed the above results (Figure 3). At 0 μ M Mn (II) the removal of colourants was low whilst at 60 μ M as well as at 165 μ M the removal was higher.

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The results obtained are consistent with the fact that Mn(II) is essential for MnP activity. The Mn(II) added to the cultures was probably used by MnP to oxidise substrates existing in the effluent, thus increasing the removal of colourants as the Mn(II) concentration was increased. Above 60 μM a "plateau" was reached, the Mn(II) added had no effect on the removal probably because there was no need for more Mn(II).

Effect of veratryl alcohol addition

In order to study the effect of manganese in the presence of veratryl alcohol, 2 mM of veratryl alcohol and various concentrations of Mn(II) were added to the effluent before the inoculation with *P. chrysosporium*.

As the concentration of Mn (II) was increased from 0 μM to 165 μM (Figure 4), there was a decrease in the fungus performance, as far as removal of colour and phenolic compounds was concerned. This decrease was even more pronounced when the concentration of Mn(II) was increased to 727 μM (Figure 5).

The results obtained in gel filtration chromatography (Figure 6) showed that in fact the removal of colourants was inhibited at high Mn(II) concentrations, confirming the above results.

Bonnarme et al. (4) and Perez et al. (27) showed that in the presence of veratryl alcohol the LiP titres varied as an inverse function of, and MnP titres varied as a direct function of, the Mn (II) concentration. Furthermore, a concentration of 727 μM Mn(II) totally repressed LiP and stimulated MnP. Therefore the decrease in fungus performance observed at high concentrations of Mn(II) could be due to inhibition of LiP and the reduction of colour and phenolic compounds observed at 727 μM would be due only to the action of MnP.

For the low Mn(II) concentrations (0, 30 and 60 μM), not inhibitory to LiP, we expected to see an increase in removal of colourants when veratryl alcohol was added, due to stimulation of LiP, as it was reported in literature. Surprisingly, we observed that the removal was always better in the absence of veratryl alcohol (Figure 1 and 4, Figure 2 and 5, and Table 1).

There is not a simple explanation for these results. Indeed, if the fungus was not producing LiP or if veratryl alcohol, in the experimental conditions used, did not stimulate LiP, the simple addition of veratryl alcohol should have a neutral, not a negative effect.

It seems that there is a synergic negative effect of manganese and veratryl alcohol.

The best results were obtained in the absence of veratryl alcohol and with a Mn(II) concentration of 60 μ M or higher (Figure 7). In these conditions we obtained 63% colour removal and 73% phenolics removal. The results from gel filtration chromatography showed an effective removal of some compounds.

CONCLUSIONS

Manganese had a positive effect on the removal of sugar colourants from the effluent by *P. chrysosporium*, but only in absence of veratryl alcohol. When veratryl alcohol was present the effect of manganese was negative.

The addition of veratryl alcohol gave rise to a decrease in the fungus performance. In terms of industrial application this is a positive result because this alcohol is very expensive.

The best results were obtained in the absence of veratryl alcohol and with 60 μ M of Mn(II). In these conditions *P. chrysosporium* was able to reduce colour by 63% and phenolic compounds by 73%.

In a near future, pilot scale experimentation will be initiated.

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The authors wish to thank to Prof. J. Lema for supplying the fungus, to STRIDE for supporting this work, to JNICT for the grant conceded to C. Guimarães, and finally to the RAR staff.

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Table 1. Colour reduction in presence or absence of veratryl alcohol (V.A.)

Conc. Mn (II) (μ M)	0	30	60
Without V.A.	51.0	57.7	62.6
With V.A.	46.5	41.7	36.9

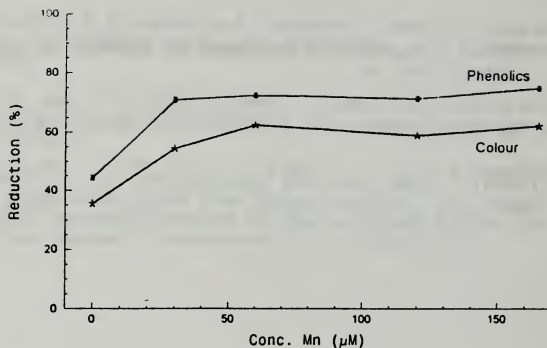


Figure 1 - Reduction of colour and phenolic compounds obtained after treatment with *P. chrysosporium* in presence of different Mn(II) concentration (0, 30, 60, 120 and 165 μM), without veratryl alcohol.

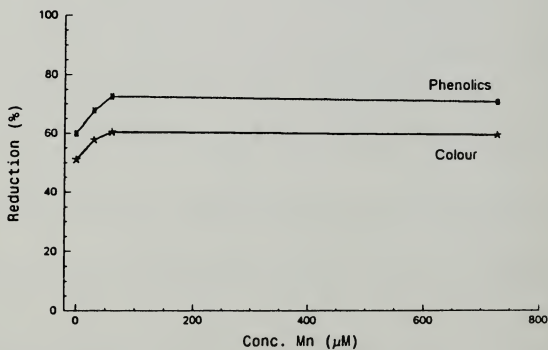


Figure 2 - Reduction of colour and phenolic compounds obtained after treatment with *P. chrysosporium* in presence of different Mn(II) concentration (0, 30, 60 and 727 μM), without veratryl alcohol.

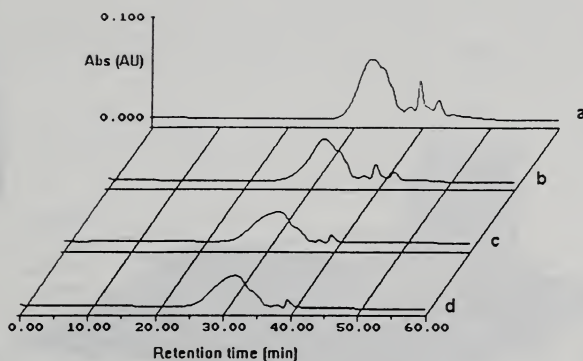


Figure 3 - Gel filtration chromatograms, at 420 nm, of the effluent not treated (a) and treated by *P. chrys* in presence of different concentrations of Mn(II) : b) 0 μM; c) 60 μM and d) 165 μM.

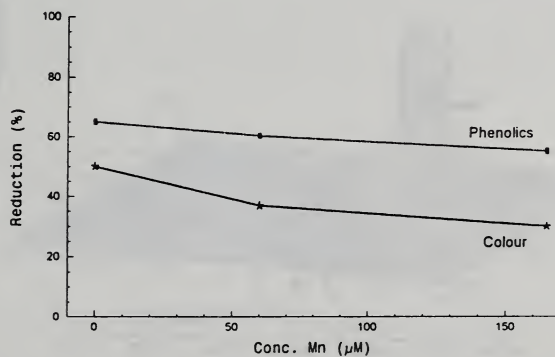


Figure 4 - Reduction of colour and phenolic compounds obtained after fungal treatment in presence of 2 mM veratryl alcohol and various Mn(II) concentration (0, 60 and 165 μM).

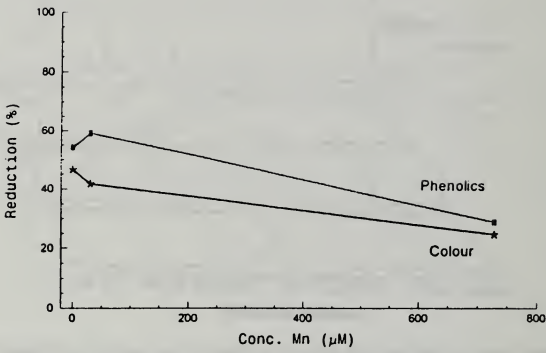


Figure 5 - Reduction of colour and phenolic compounds obtained after fungal treatment in presence of 2 mM veratryl alcohol and various Mn(II) concentration (0, 30 and 727 μM).

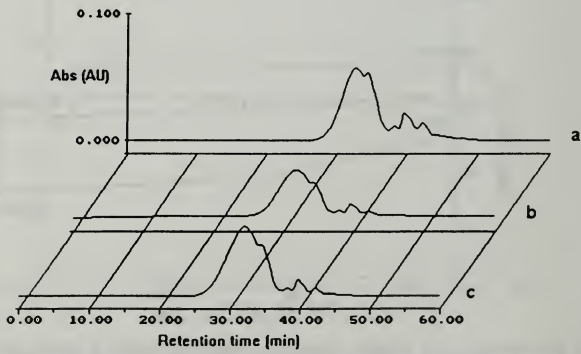
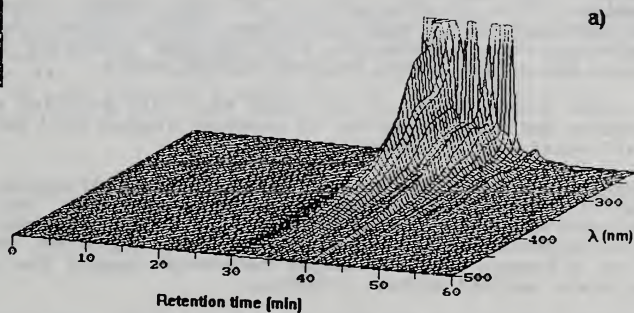


Figure 6 - Gel filtration chromatograms, at 420 nm, of the effluent not treated (a) and treated by *P. chrys.* in presence of 2 mM veratryl alcohol and 0 μM (b) or 727 μM (c) of Mn(II)

Abs (AU)



Abs (AU)

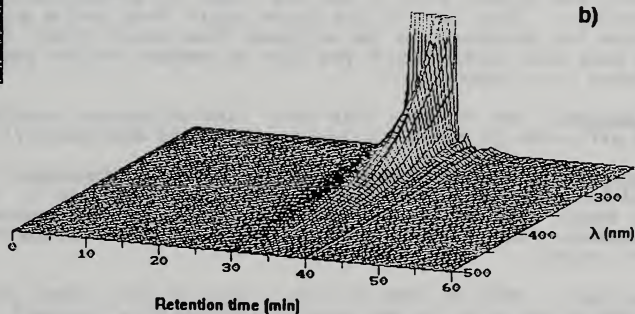
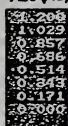


Figure 7 - Diode array gel filtration chromatogram of the effluent before (a) and after (b) treatment with *P. chrysosporium*, in presence of 60 μ M of Mn(II), without veratryl alcohol.

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DISCUSSION

Question: Two questions: one, how long do these fermentations take to get to, say, 60% color removal.

Guimaraes: A long time - about 25 days. We are now trying to get this in 15 days. However, I would like to emphasize that an anaerobic treatment lasts about 30 days.

Question: This is with a strain of *Phanerochaete chrysosporium* that produces the lignin peroxidase (LiP) enzymes only under nutrient stress. There are other strains available that will produce the enzyme before nutrient stress occurs.

The other question is, do you think that this positive effect of magnesium and negative effect of veratryl alcohol will be the same in all strains of *P. chrysosporium*?

Guimaraes: I really don't know. All the published papers say that veratryl alcohol stimulates production of LiP. But in our experiments, the addition of veratryl alcohol gives poorer results. The experimental conditions we use are quite different, because we use an effluent, and literature references report the use of a completely defined synthetic medium.

Question: Thank you for this interesting work. Are you aware, I wonder, of some work carried out in the early 1980's by Prof. Carl-Eric Ericsson at the Swedish Forest Products Research Institute (STFI) and by Kent Kirk of the U.S. Dept. of Agriculture, Madison, Wisconsin. They got to the point where they had a pilot scale system for decolorizing the effluent from paper mills. I see that you know this work. Would you like to comment on how your results compare with theirs?

Guimaraes: Yes, we know this work. The colourants profile of the two effluents is quite different. They did use veratryl alcohol.

Question: A comment: the application of the *P. chrysosporium* to the wood industry is obvious, because the product is lignin. The white rot fungi grow on wood. The application of the system to the remediation of chemical spills and to the sugar industry is, in fact, novel.

Question: What is the percent color in the first portion of regeneration which is not treatable by Bento's process?

Bento: About thirty percent. As it is observed on the special regeneration curve, we have 30% of the total colourants in the salt effluent that are separated at low salt concentration. This part of the effluent is not treated with lime.

Question: Two short questions: one comment, have you looked at any other co-factors as well as manganese - cobalt, for example, and number two, why did you measure the color at pH9?

Guimaraes: To answer the second first, because SPRI's papers recommend a pH higher than 7 as the most stable pH to measure color. On the question of other co-factors, we published a study on the effects of pH, temperature and other factors, (Int. Sugar J., 95: 339-343 (1993)) but we did not look at other co-factors - manganese is well known as a co-factor for these peroxidases.

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APPLICATIONS OF FOURIER TRANSFORM INFRARED SPECTROSCOPY
TO THE IDENTIFICATION OF WHITE SUGAR CONTAMINANTS

D. Eugene Rearick

The Amalgamated Sugar Company, Twin Falls, Idaho USA

ABSTRACT

Fourier transform infrared spectroscopy is a powerful tool for the identification of white sugar contaminants. In addition to conventional infrared sample preparation methods, very small samples can be analyzed using beam masks and microscopic mounting techniques to avoid the need for more costly infrared microscopy equipment. Examples of applications to be given include the identification of plastic fragments, inorganic constituents, paint chips, and components of homogeneous mixtures with sucrose.

INTRODUCTION

For nearly 50 years mid-range infrared (IR) spectroscopy has been routinely used for the identification of compounds containing covalent bonds. This spectral region (2.5 to 20μ or 4000 to 500 cm^{-1}) provides information on the types of chemical bonds present in a substance as well as frequently showing multiple peaks which can serve as a "fingerprint" for comparison of two materials.

IR spectroscopy is invaluable in the identification of white sugar contaminants and two earlier papers have described its use in our laboratory (1,2). This work utilized IR spectra recorded on an older dispersive instrument to identify rubber particles and inorganic contaminants such as pan scale. Dispersive IR spectrometers have lower signal to noise ratio and beam power than modern Fourier transform infrared (FTIR) spectrometers and thus cannot be used directly on difficult samples such as whole fibers or polymer fragments. The relatively low-cost FTIR spectrometers, which have become available in the last few years, can be applied to a variety of sugar industry problems requiring the characterization of sugar contaminants, insulation, process scale, lubricants, and packaging materials. The application of FTIR to the identification of fibers in both sugar samples and insulation has been described previously (3). This paper gives several examples of the application of FTIR spectrometry to identification of a variety of contaminants in white sugar.

EXPERIMENTAL

All infrared spectra were recorded on a Perkin-Elmer 1650 FTIR spectrometer, equipped with a DTGS (deuterated triglycine sulfate) detector, at 4 cm^{-1} resolution. Fiber and polymer samples were analyzed directly using a Perkin-Elmer 1700 microfocus attachment and a 1000μ circular aperture beam mask. Other solid materials were analyzed by a potassium bromide (KBr) pellet technique as noted.

RESULTS AND DISCUSSION

A. Identification of plastic fragments

Plastic particles are occasionally introduced into sugar at either the producer's or customer's location. Such contaminants can originate from several possible causes including abrasion of plastic process equipment components and abrasion of foreign objects, such as plastic containers, accidentally introduced into equipment. Usually such materials are detected on a customer's screen or, in the case of liquid products, a check filter but occasionally may be observed on sediment pads. Obviously, the identification of any such material is the first step in locating a possible source. Since thin plastic samples give very well-defined and characteristic IR spectra directly, the only sample preparation usually necessary is obtaining a thin slice of material.

An example of the application of FTIR to plastic identification was provided by a customer sample containing several of what were first thought to be dark-colored fibers found in bagged sugar. The particles were only 1-2 mm long and approximately 0.1 mm in diameter. Under the microscope the particles were observed not to have a typical fiber structure but were simply elongated fragments or shavings of a plastic material or possibly a dark-colored wood. A single particle was, under the microscope, carefully picked up with tape on one end and placed across the aperture of a 1000μ beam mask. The IR spectrum was recorded using a microfocus attachment (beam condenser). The spectrum, shown in Figure 1 indicated the material not to be cellulose, nylon, polyester, polyethylene, or any of the other common polymeric materials in our spectral library. (The elimination of cellulose as a major constituent also precluded the possibility of the material being wood.) Reference to a published spectral library (4) indicated the three strong bands at 917 , 1094 , and 1236 cm^{-1} to be characteristic of the ether carbon-oxygen bond stretching frequency in an acetal resin. In particular, the spectrum is very similar to that of Dupont Delrin 500 acetal resin. Acetal resins are strong, stiff thermoplastics

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with good temperature resistance and are typically used in molded electrical and mechanical parts such as pump impellers, conveyor links, or drive sprockets (5). It seems likely that these particles were abraded from some type of process equipment. In this case, the source of the particles was unfortunately never located but at least the problem did not reoccur.

A second example of polymer identification was the use of FTIR to identify a white, shredded material found on a customer's screen. The IR spectrum of a thin fragment, shown in Figure 2, is the typical hydrocarbon spectrum of polyethylene. Polyethylene is used for such a variety of applications that its identification does not narrow down the search for a contaminant source very much. In this case sugar handling equipment at the shipping location was found to have some polyethylene components but the distinct possibility remained that this material could have come from other sources such as a polyethylene sample container or retail product container introduced into bulk sugar at any location and shredded by sugar handling equipment.

Finally, a small fragment of a plastic film, about 1 mm x 3 mm in size, was sent to us by an individual retail customer. The principal question was whether or not this material, found in a paper sugar bag, was a fragment of the polyethylene bagging film present at the location that the sugar was packaged. Under the microscope, the fragment was found to be coated with an adhesive on one side. Adhesive was scraped off with a needle and an IR spectrum of the plastic film was recorded directly. The spectrum, shown in Figure 3 (upper spectrum) contains peaks at 1426, 1331, 1255, and 614 cm^{-1} characteristic of the carbon-hydrogen and carbon-chlorine absorptions of polyvinyl chloride (PVC). A spectrum of a known PVC sample is shown at the bottom of Figure 3. The identification of PVC, plus the presence of an adhesive, suggested that this sample was not polyethylene bagging film but more likely a fragment of plastic tape, possibly from the customer's home.

B. Identification of metal salts and inorganic contaminants

FTIR spectrometry, as mentioned previously, can be used in the identification of very small samples of material, including insoluble particles recovered from sample filtration in sediment tests.

In an example of this application, a 500 g sample of sugar said to be high in sediment was dissolved in water and filtered through a white sediment pad. Examination of the filter under the microscope showed a number of small tan or brown flakes, some with black layers. The entire amount of insoluble material (1 mg or less) was transferred to a mortar, finely ground with potassium bromide, and

pressed into a pellet. The infrared spectrum of the resulting sample is shown in Figure 4 along with a reference spectrum of calcium oxalate. The identical spectra indicate that the insoluble particles were probably an oxalate pan scale.

Another example was provided by liquid sugar containing a sediment. Filtration of 2.5 liters of liquid sugar through a black sediment pad, followed by water washing and drying of the black pad, gave a small amount of very fine, colorless needles. The IR spectrum of this material, as a KBr pellet, showed it to be crystalline calcium carbonate (Figure 5). Presumably in this case the contaminant was due to hard water used in syrup preparation.

A final example of inorganic contaminant identification arose from an individual customer who returned a sugar sample containing translucent beads 2-4 mm in diameter. These beads were glassy in appearance but much more easily fractured than true glass. The IR spectrum of this material, in KBr, showed an intense absorbance at 1082 cm^{-1} with only two other significant peaks (952 and 790 cm^{-1}) (Figure 6). Such simple spectra are indicative of a simple, covalently bonded compound, possibly inorganic, and intense peaks in the 1000-1100 cm^{-1} region of the spectrum are typical of silica or silicates and phosphates. The general appearance of the solid plus the presence of a light pink color in some beads suggested that the material was a silica gel desiccant with a cobalt chloride indicator. Comparison of the spectrum with one of a known silica gel sample confirmed this and additional confirmation was provided by heating the beads to give the bright blue color typical of a cobalt chloride indicator. In this case the source of the contaminant was not found. The single occurrence suggests that it likely originated accidentally in the customer's home although the possibility of a single deliberate contaminant addition could not be ruled out.

C. Characterization of homogeneous mixtures of sugar and a foreign material

Most cases of sugar contamination are due to obvious particles either detected by direct observation or filtration; however, sugar is occasionally contaminated with white powdered or granular materials that give essentially homogeneous mixtures. In our experience such instances have always been due to accidental mixing of sugar with other products by a customer and have involved mixtures with materials such as salt, powdered cream substitutes, or sodium borate (borax). Since sugar is still usually the major component of such mixtures the use of IR spectrometry directly on the mixture would give essentially just a sucrose spectrum. For this reason, the contaminant must first be isolated from the mixture. If there are obvious differences in crystal shape or size

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between components, the small amount of material necessary for an IR spectrum may be physically separated under a microscope. This technique was used in the case of contamination with sodium borate discussed previously (2).

In a more recent example of a homogeneously contaminated sugar sample, no obviously different particles were visible under the microscope. The sugar in question had been in use at a food stand and was said by the establishment operator to have a bitter taste but also exhibited an excessively sweet aftertaste. Depending on the individual, both bitter flavors and sweet aftertastes are characteristic of artificial sweeteners containing saccharin. The presence of sodium and calcium at significant levels (200-400 ppm) and a pol value of +108°Z also agreed with the presence of a commercial artificial sweetener since such products can contain these cations and often contain maltodextrin, which has a specific rotation more positive than sucrose. Evidence of the presence of saccharin was provided by acidifying a normal weight solution of the sample, extracting with diethyl ether, drying the extracts and evaporating to a solid, according to AOAC Method 941.10 (6). While the AOAC method specifies tasting the residual solid, more conclusive evidence was provided by the upper IR spectrum shown in Figure 7 which is rich in distinctive fine structure and identical to the spectrum obtained by treating an authentic saccharin sample in the same way (lower spectrum). It is strongly suspected that in this case the sucrose had been on an open counter, accessible to customers, and that accidental mixing of sugar with an artificial sweetener had occurred.

D. Comparison of paint particles

Paint flaking from various sugar handling surfaces is a common problem and can occur anywhere from sugar end equipment through storage, shipping, and a customer's unloading equipment. Evidence of contamination from flaking paint usually is encountered in sediment tests on granulated sugar or check filters in the case of liquid products. Paint flakes can usually be identified as such by microscopic examination but valuable information about paint composition can be obtained by IR spectrometry and ultimately the spectrum may be compared with those of paint samples from suspect surfaces to verify the origin of the problem. Well-equipped forensic laboratories that routinely examine paint chips do so by slicing paint specimens with a microtome and analyzing various areas of a cross-section with FTIR microscope equipment. In this way, individual layers of multiple-coating paint chips may be identified. In the absence of such equipment, good IR spectra of paint samples may be obtained by grinding a small fragment with potassium bromide and pressing the mixture into a disk. Care must be taken to carefully examine the fragment under the microscope to ascertain whether it is a single paint layer that can be used to

obtain information about composition or whether it is a multiple-coat specimen. In the case of multiple paint coats the IR spectrum may still be useful as a fingerprint to compare with other samples but may not provide useful information on paint composition.

An example of the application of IR spectrometry to paint identification occurred recently in our laboratory when a customer recovered white paint chips from a liquid sugar filter. The customer evidently had no painted sugar handling equipment and the sample did not have the characteristic color of coatings in railcars used by our company. The material was thus thought to have come from the shipping location. In this case, samples received appeared to be a thick, homogeneous, single paint coating and a 1-2 mg sample pulverized with KBr gave the spectrum shown in Figure 8. A brief manual search of a published polymer library showed that the spectrum has several peaks (1508 , 1458 , 1248 , 1182 , and 828 cm^{-1}) which are characteristic of a bisphenol-A/epichlorohydrin epoxy resin such as Shell Chemical Epon Resin 840 (7). Although spectra of epoxy resins have a sharp peak at 1010 - 1020 cm^{-1} the 1020 cm^{-1} absorbance of the paint sample is much too intense and is thought to be due to a silica-based pigment.

Paint samples from seven surfaces around the sugar storage and loading areas of the shipping location were examined by the same technique. Most of these proved to be alkyd enamels rather than epoxy coatings and were in fact distinguishable from each other due to differences in resin or pigment composition. Figure 9 shows the IR spectrum of a typical alkyd enamel. Note the intense ester carbonyl bond absorbance at 1736 cm^{-1} and carbon-oxygen single bond absorbance at 1262 cm^{-1} which are typical of the polyester structure of alkyd resins. Peaks at 1120 and 1068 cm^{-1} are also typical of common alkyd resins (8). The intense absorbance at 1420 cm^{-1} and sharp peaks at 876 and 713 cm^{-1} are not part of an alkyd resin spectrum but are characteristic of calcium carbonate, evidently a component of the pigment system in this paint. The broad intense band at approximately 500 - 700 cm^{-1} is the only IR absorbance (in this range) of titanium dioxide which is the major covering agent in many modern paints. Note that calcium carbonate is not detectible in the contaminant paint (Figure 8) although the broad titanium dioxide band could be present.

Finally two epoxy coatings which had been used in different sugar silos were examined by IR spectrometry. The first sample (Figure 10) shows several of the peaks due to an epoxy resin (1510 , 1458 , 1250 , 828 cm^{-1}) but is substantially different than the complaint material. Note that the pigment still appears to be silica-based but the peak maximum (1093 cm^{-1}) is substantially different than the sample from sugar and additional peaks (799 , 779 , 694 , 464 cm^{-1}) are present that are not in the contaminant spectrum. Although an

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epoxy coating, this material is evidently not the source of the problem. The second epoxy coating, which had been used in a single silo, gave the spectrum shown in Figure 11. This material is different than the first epoxy and its IR spectrum is virtually identical to the complaint sample, with a silica-based pigment peak at 1020 cm^{-1} . This IR spectrum provides strong evidence that the contaminant paint came from this particular sugar silo.

SUMMARY AND CONCLUSIONS

Fourier transform infrared spectrometry is an extremely valuable tool in determining the identity and source of a variety of white sugar contaminants. Contaminant materials originating in or around storage, loading, transportation, or unloading equipment can often be characterized and compared with known samples by this method. In addition, substances accidentally mixed with sugar by customers can frequently be identified thus avoiding more serious questions of product contamination. FTIR analysis requires only a small sample and can be carried out with one milligram or less of material, an amount which can be provided, for example, by a few particles on a sediment test filter pad, a few fibers, or a plastic fragment approximately 1 mm across. Materials identified or characterized using FTIR can include polymers, synthetic or natural fibers, paint chips, process scale constituents, and food ingredients other than sucrose but practically any contaminant separable from sucrose can be at least partially characterized by the method.

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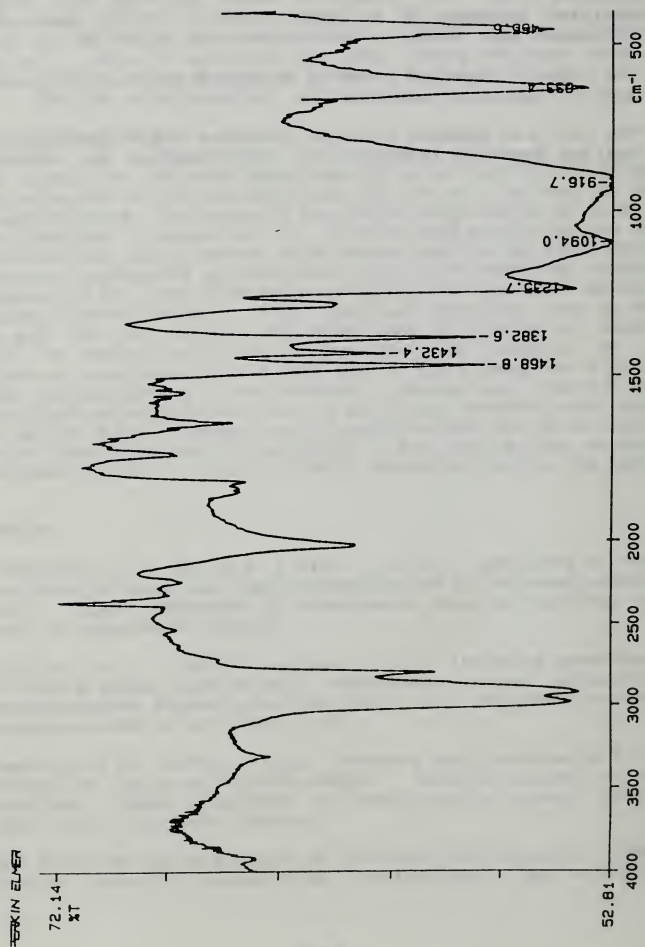


Figure 1. Dark fibers in white sugar.

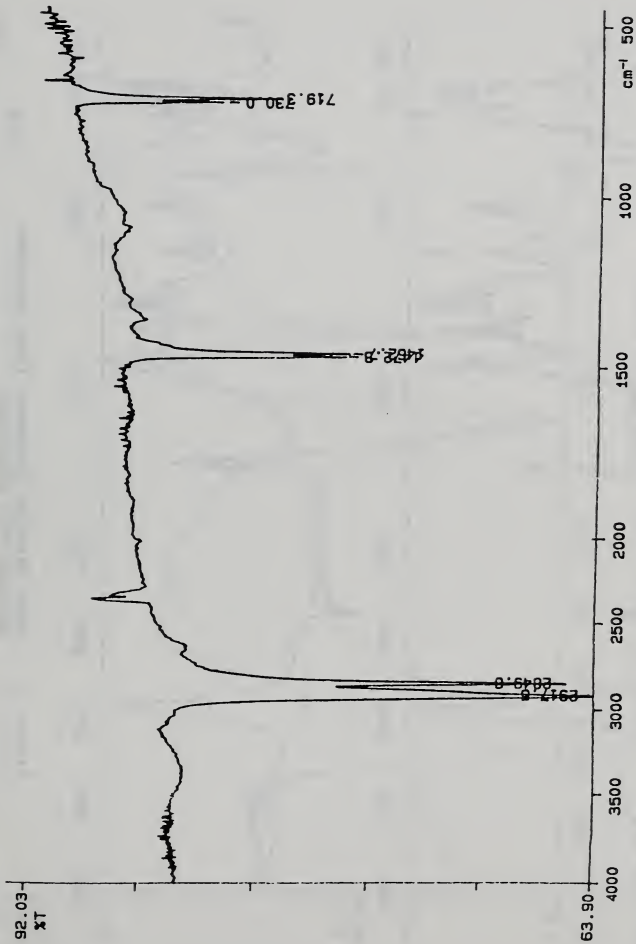


Figure 2. Plastic fragment from screen.

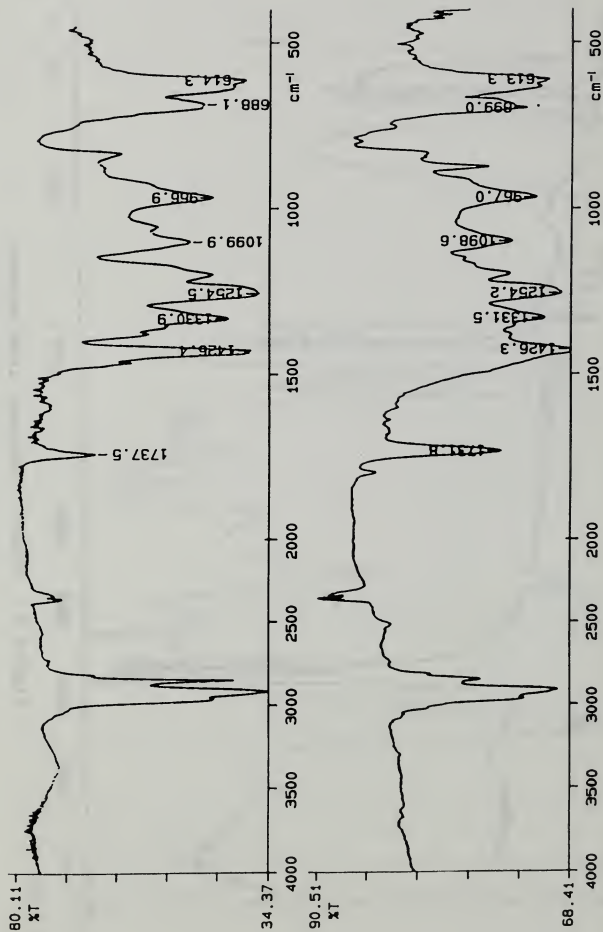


Figure 3. Top: Plastic fragment from customer
Bottom: PVC spectrum

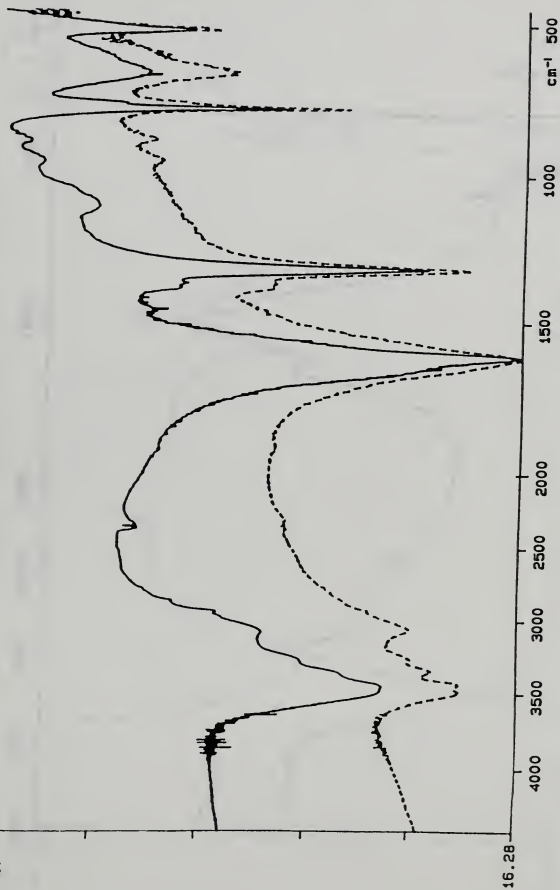


Figure 4. Solid line: Specks from sugar
Dotted line: Calcium oxalate

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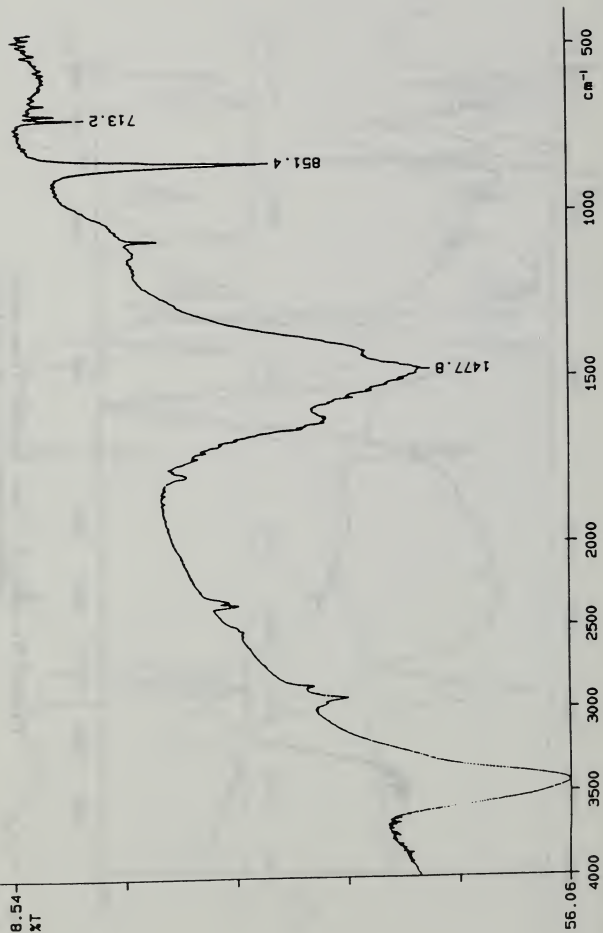


Figure 5. Small needles from a liquid sugar.

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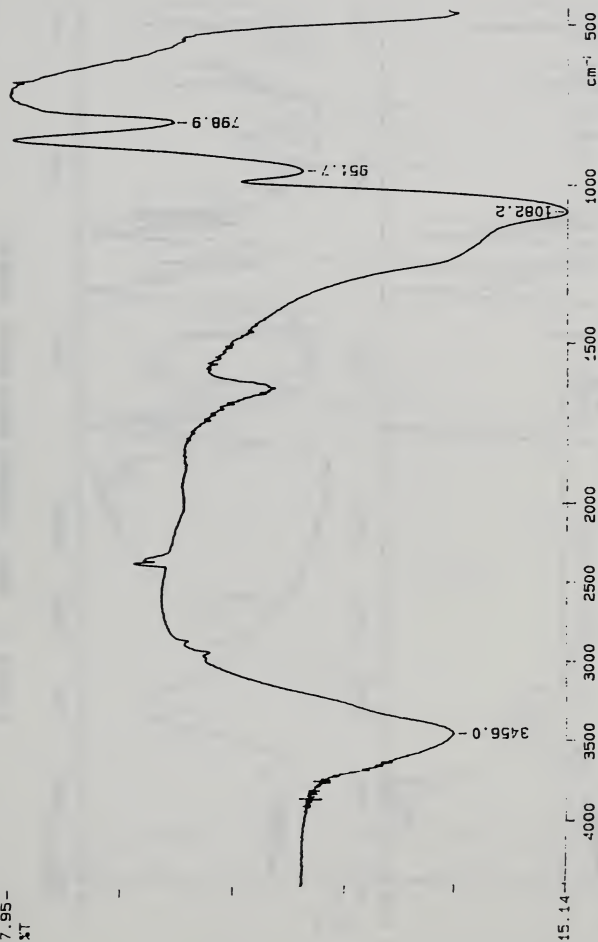


Figure 6. "Beads" from white sugar.

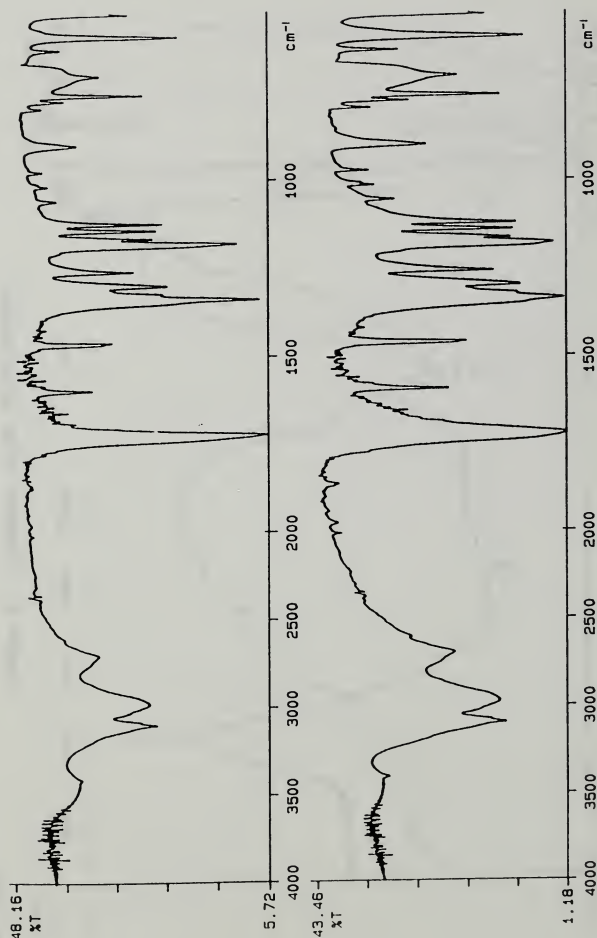


Figure 7. Top: residue from sugar sample.
Bottom: Saccharin.

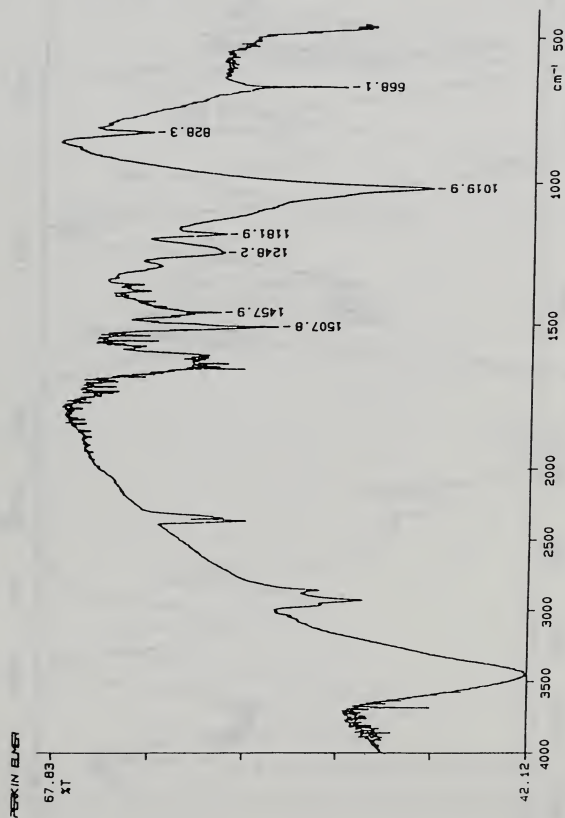


Figure 8. Paint chip from customer

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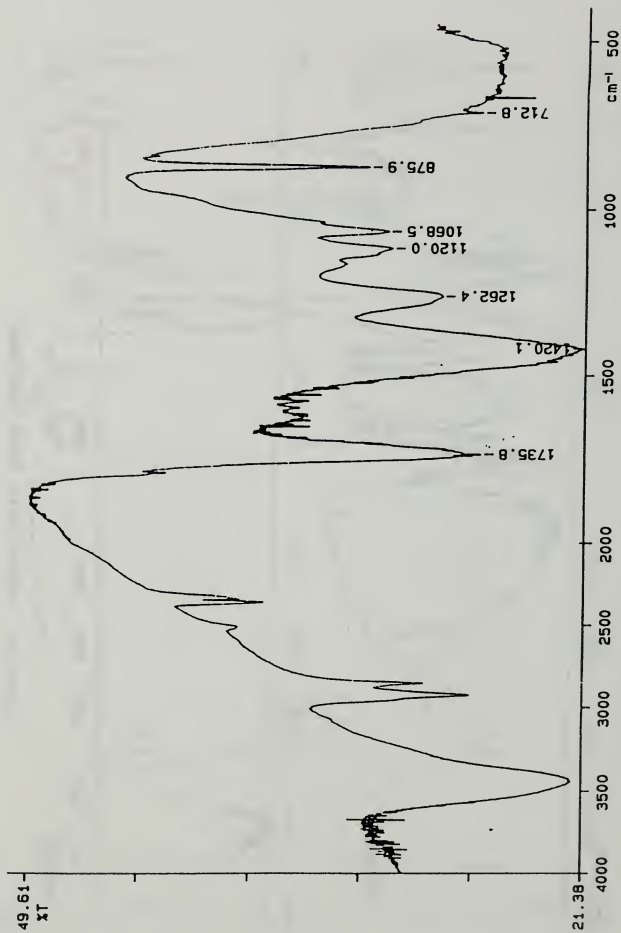


Figure 9. Paint flake, under loading scroll

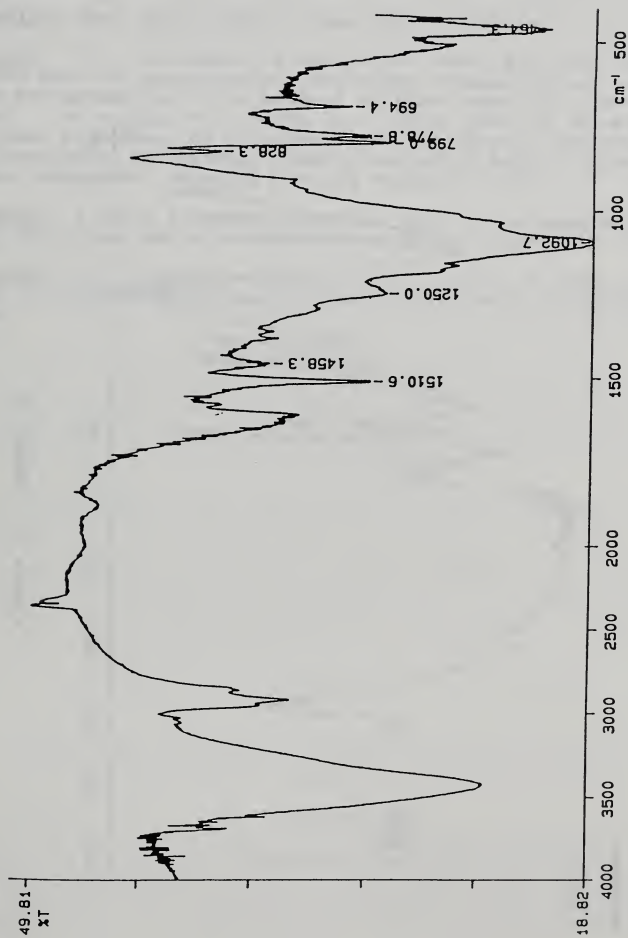


Figure 10. Epoxy #1

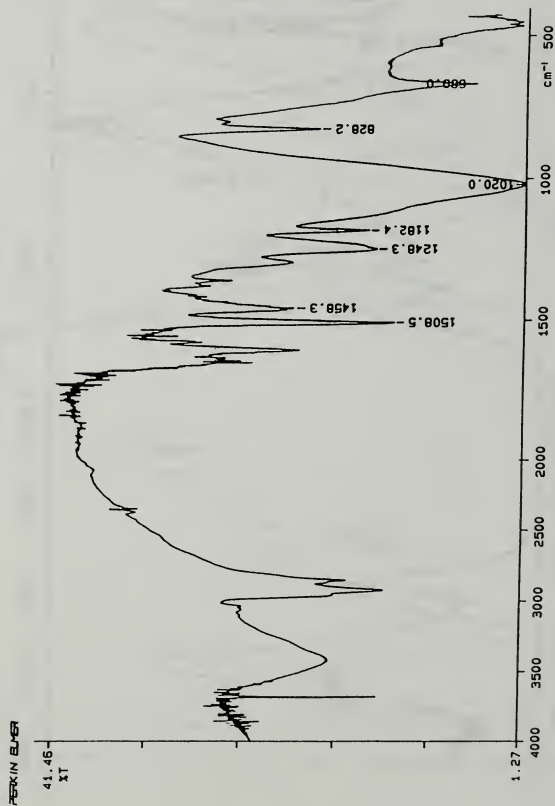


Figure 11. Epoxy #2

DISCUSSION

Question: What is the cost of this sort of system?

Rearick: The instrument we bought was about \$16,000, with about \$1,000 more for the plotter. The other accessories are not much - beam condensers are a few hundred dollars. About \$17,000 - \$18,000 total. It's been well worth it - we've used it to solve many customer problems, and for other uses - to identify asbestos around the factories, for example. Identification of bagging films, and of scale components, make it a really valuable tool.

Question: A small technical question: what is the sample presentation for films and solid materials - do you grind them up?

Rearick: No. I showed a beam mask in a slide. We put a piece of film, or a small solid sample directly on to that, securing it with tape.

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Helsinki, Finland, August, 1994

AUTOMATIC ANALYSING OF SUGARBEET AND SOIL SAMPLES IN FINLAND

Marja Pelo¹ and Harri Pietinen²

¹Sugar Beet Research Centre, 25170 Kotalato, Finland

²Akasystems Oy, Heinämaantie 6, PI 20, 40251 Jyväskylä, Finland

ABSTRACT

Finland is the northernmost country where sugarbeet is grown. The short growing period forces the harvest of immature sugarbeets. Therefore the beet quality plays a significant role. The payment to the farmers is since 1987 based on recoverable sugar.

The analysing of all sugarbeet samples from the three Finnish sugar factories is centralised to Sugar Beet Research Centre, where all the quality values have been analysed since 1980 with two Betalyser-analysers. About 100,000 frozen and vacuum packed samples per year are handled with warm lead acetate solution. The analysis includes pol, sodium, potassium and amino-nitrogen contents.

Since correct use of fertilizing is the foremost precondition for good beet quality, the analysing of soil samples and calculation of fertilizer recommendations are concentrated in the same laboratory. Soil samples are analysed with a third Betalyser-analyser after extraction with ammonium acetate solution and pH is measured from soil-water-suspension. The number of soil samples exceeds 10,000 samples/year. The analysis includes pH, phosphorous, sodium, potassium and magnesium.

Betalyser systems, of 15 years service, were equipped with program made by Basic-language.

The old control system of the Betalyser has now been replaced by PC-based technics. No changes to the instruments were needed. The modernising work was made by Akasystems Oy from Finland.

The analysing data is stored in the database of the computer for further processing. The programs are based on C-language.

User-interface contains pull-down menus. A HELP system provides the user with on-line information, which is most important when using seasonal workers.

Each step of the processing sequence is shown continuously on the screen. After each complete sequence the analysis results are shown on the screen and printed. Furthermore, the results are stored in the daily databases.

One analyser unit makes it possible to analyse 120-150 beet or 60 soil samples per hour.

INTRODUCTION

The analysing of all sugarbeet samples from the three Finnish sugar factories is centralised to the Sugar Beet Research Centre. The analysing of soil samples and calculation of fertilizer recommendations are also concentrated to the same laboratory.

The laboratory is fully automated and the analysing of both sugarbeet and soil samples will be made by three Betalyser systems. These Betalysers (two from 1980 and the third 1990) are bought from Optik Elektronik Automation Dr. Kernchen, West Germany.

Circa 50 Betalyser systems are sold to different parts of the world (for sugarbeet and sugarcane analysis) mainly to Europe for sugarbeet analysing. Only a few Betalysers are equipped with soil program.

The analysing system contains several analytical instruments, peristaltic pumps, solenoid valves, computer with printer, floppy disks and bar code reader.

The biggest advantage of this kind of system is the longer usage time of instruments. Extra reliability of instruments, computers and pumps is needed during campaign. The best result is obtained with spare instruments which can be in use with soil samples. The amount of these analysis is not so critical and they can wait for repairing of instruments. The service costs with several instruments of same type are low.

One Betalyser-unit is in use circa 8 months/year and the other two during the campaign. Some of the skilled seasonal persons can work for longer time and take care of the regular services. This is also an economical point.

Betalysers of more than 10 years service were equipped with old computers and the program was made by Basic-language. All other instruments were still working satisfactorily. For the reasons above we decided to start development project together with Akasystems Oy from Finland. The goals of the development project were to replace the old computers and programs on the following terms:

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- New compatible PCs replacing the old computers
- No changes to the instruments and the peripheral devices needed
- Increased reliability of data saving
- Improvement of user-interface (i.e. user-friendliness)
- Easier further processing of measurement data

As a result of this co-operation, analysing systems called "SOKRU" and "MUDDY" were completed in the spring 1994. Later in the summer 1994 a new fertilizer recommendation program was also completed. This is one example and modification of laboratory automation work made by Akasystems Oy.

MATERIALS AND METHODS

Pretreatment of sugarbeet samples

25 Kg of sugarbeet out of every truckload are washed, sawed and homogenised in the factory. 60 g of brei is put into a gas- and water-proof plastic bag and marked with two bar code labels for parallel analysis when necessary. Bags are closed with vacuum packing machine and quickly frozen and then transported in thermo boxes to Sugar Beet Research Centre.

Frozen samples are homogenised with warm lead-acetate solution and filtrated. The pollution problem is solved with precipitation with calcium carbonate.

Two Betalysers are used during campaign for sugarbeet quality analysis: sugar, sodium, potassium and amino-nitrogen are determined from every sample.

About 100,000 samples are analysed during two months in two shifts (120 samples/hour with one analysing unit). Altogether 8 seasonal workers (4/shift) handle and analyse all these frozen sugarbeet samples.

Pol- λ is determined with a Sucromat polarimeter; sodium and potassium with IL-flame photometer and amino-N with Testamin-photometer, by Blue number method.

Pretreatment of soil samples

Farmers or advisers take soil samples and send them by post to Sugar Beet Research Centre. Here they are dried, milled and extracted with acidic ammonium acetate solution. The pH is measured from soil-water-suspension. The handling of solutions is also automated with Kemex dosing systems developed by Akamex Oy from Finland.

The fertilizer recommendation is based on information of protocol, soil type and humus classification and analysed values of pH, sodium, potassium, magnesium and phosphorous. The analysed micro nutrients are copper, zinc and manganese.

The measuring system for soil samples is mainly the same as the one for sugarbeet samples. Part of the analytical instruments has been changed. Sodium and potassium are again measured with IL-flame photometer, phosphorous with Testamin-photometer (water bath), magnesium with Atomic Absorption Spectroscopy (AAS) and pH with pH-meter. The micro nutrients are measured with AAS.

The analysing of soil samples takes 40 seconds compared to 20 sec with beet samples. The development of colour is time taking in phosphorous measurements is the time extension cause.

The amount of soil samples exceeds 10,000 samples/year. About 2000 fertilizer recommendations are calculated every year.

Data handling of sugarbeet and soil samples

The programs are modified by Akasystems Oy from Finland and they are now based on C-language. The user-interface contains pull-down menus. A Help provides the user with on-line information, which is important to seasonal workers.

Each step in processing sequence is shown continuously on the screen. After each complete sequence the analysis results are shown on the screen and printed. Furthermore the analysis data is stored in the daily database in the computer for further processing.

Daily sugarbeet results are sent to the factories with modem and statistical parameters are calculated and printed weekly on daily databases.

The soil analysis results are connected to farmers' database and the protocol information as far as the fertilizer recommendation is calculated for sugarbeet cultivation. Graphics and statistics are also connected to fertilizer recommendation program.

SUMMARY

Finland is the northernmost country where sugarbeet is grown. The short growing period forces the harvest of immature sugarbeets. Therefore the beet quality plays a significant role. The payment to the farmers is, since 1987, based on recoverable sugar. All quality values have been analysed since 1980 in Sugar Beet Research Centre.

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Since correct use of fertilizing is the foremost precondition for good beet quality, the analysis of soil samples and calculation of fertilizer recommendations are concentrated in the same laboratory.

The development of both new economical analysing systems and new quality analysis for beet and soil are important. Looking to the future in the laboratory the next step is to connect all 3 analysing PCs together with main PC network. This gives a possibility to an automatic data transferring from soil analysing station to the fertilizer recommendation program. Centralised data handling for beet samples is another advantage network connection gives.

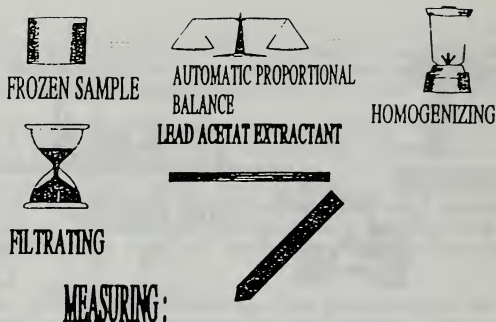
The conductivity and calcium measurements from soil samples are the second analysis which will be connected to the system. There are no technical limitations in connecting new instruments.

The glucose and dry matter values in beet analysis can be stored to the database with network. They are now stored manually. The connection to new instruments, e.g. NIR, is now possible.



Figure 1. Preparing of sugarbeet samples

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VALUE: INSTRUMENT:
POL % SUCROMAT
POLARIMETER
K, Na IL543
FLAME PHOTOMETER
 α -N- TESTAMIN PHOTOMETER
BLUE-NUMBER-METHOD
(CuNO_3 + Na-ACET.BUFFER)

CALIBRATION:

POL %: WITH D.I. WATER

K, Na: WITH D.I. WATER + ONE STANDARD

α -N: WITH D.I. WATER

(LOG. CALIBR. CURVE STORED)

Figure 2. Pretreatment of sugarbeet samples

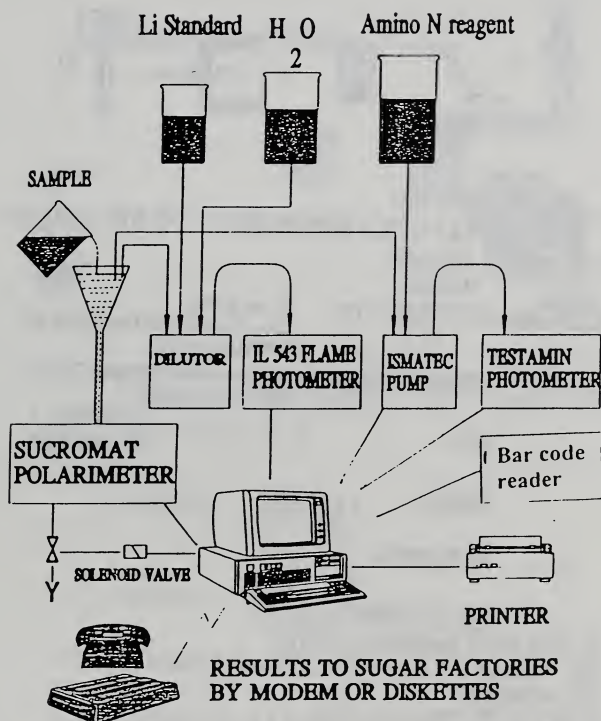


Figure 3. Betalyser computerized analyser for sugarbeet quality determination.

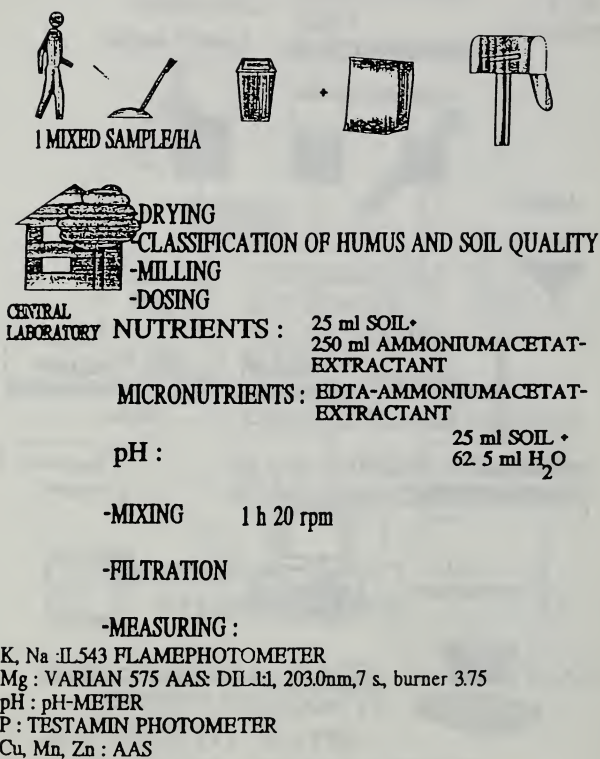


Figure 4. Preparing of soil samples

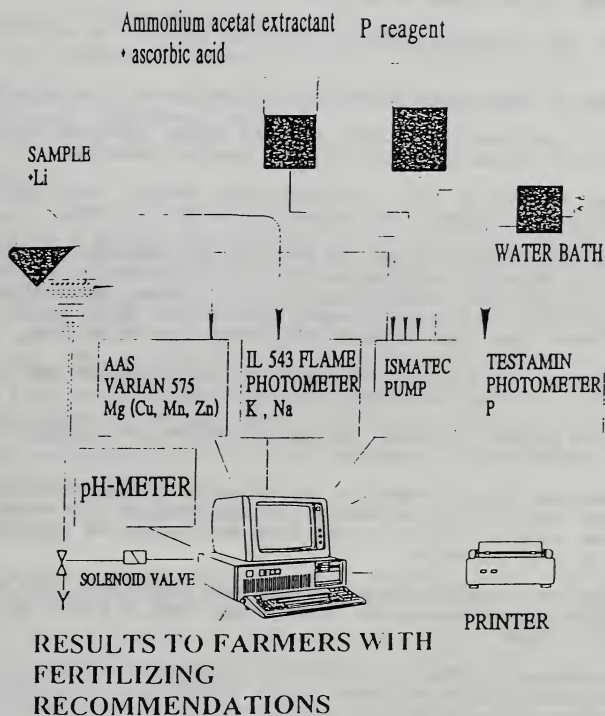


Figure 5. Betalyser computerized analyser for soil samples

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DISCUSSION

Question: This is a very nice service to your farmers, to analyze the soil samples. I wonder if you'd comment on why you are not analysing the nitrogen in soils?

Pelo: We don't measure nitrogen on every sample - we may do this in future.

Question: By what means do you analyze nitrogen - by an electrode?

Pelo: We have analyzed trial samples using ammonium and nitrate electrodes, but we don't do this on regular samples.

Question: How have you validated your system, and what is the uncertainty, or error, in the results of the system.

Pelo: We use control cards. We put control samples into the system every five minutes, get the results into the computer and so follow the level of each analysis - sugar, sodium, potassium, amino nitrogen, etc. The government authorities also check on this system each year, and we send the control cards to them.

Question: So, it's not necessary to give an error estimation to the customer - you can just use the control charts?

Pelo: The payment is based on quality values, so we can't send the error values to the customers.

If something appears wrong - for example, if polarization values are very high or low - we make a parallel analysis to check. Using the same control solution with both Betalysers we can follow the level of each analysis.

Question: You mentioned that you use lead acetate in your analyses. Is there any pressure to stop using lead acetate and to use something else?

Pelo: We have not changed to aluminum sulfate. We precipitate 99.9% of the lead acetate and send it to treatment. Of course we are encountering some demand to change - but then we'll have to change instruments too. However, Dr. Kernchen also supplies instruments for use without lead acetate.

Question: The soil analysis is a rather new system here - have you any evidence for farmers improving their soil quality for beet because of your results?

Pelo: Yes, the beet quality in Finland has improved over the last 10 years, since this program began.

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Helsinki, Finland, August, 1994

HEAVY METAL CONCENTRATIONS IN SUGARBEET FIELDS AND SUGARBEET IN FINLAND

Matti Erjala¹ and Raimo Ervio²

¹Sugar Beet Research Center of Finland, Perniö, Finland

²Agricultural Research Centre of Finland, Institute of Soils and
Environment, Jokioinen, Finland

ABSTRACT

The heavy metal (Cd, Cr and Pb) study of Finnish sugarbeet fields and sugarbeet of 1993 shows that the sugarbeet fields are as clean as any other surrounding fields from heavy metals within the same region in Finland. The heavy amelioration of sugarbeet fields has not had any direct negative influence on the heavy metal concentrations in the soil. The heavy metal contents in Finnish sugarbeet fields are rather low as compared with the figures from other countries. The sugarbeet root is quite free from heavy metals, whereas the tops seem to be good absorbers of cadmium. The rather high concentrations in the tops can be lowered in future by further liming of the sugarbeet fields and by the use of phosphates low in cadmium. The lead content in the tops can be fairly high locally, but generally it is low. Chromium is found moderately in the tops.

INTRODUCTION

Environmental issues are of great concern today among people involved in agriculture. A number of authors in Finland and abroad have shown how man's activities leads to enrichment of the biosphere with originally rare elements. In this respect, great attention is drawn to the accumulation of heavy metals in the soils and plants, because for the well-being of plants, animals and humans the matter is vital. Lead is a major chemical pollutant of the environment and highly toxic to man and animals. Cadmium is toxic both to plants and animals. Chromium appears to be essential to man and animals (6). Chromium toxicity effects have been observed however in oats (7).

Heavy metals are emitted into the air from energy production, traffic and industry. Part of the emission is deposited onto the cultivated fields and plants. In Finland, fields are naturally acid and nutrient poor. To improve the growth potential of the

fields, they have required intensive amelioration, and this has been done by applications of limestone powder, superphosphate, potassium salt, etc., in some cases of sewage sludge. Especially the use of superphosphate and sewage sludge can be crucial for the contamination of soil with heavy metals. Superphosphate may have had some influence on the cadmium status of sugarbeet fields. It has been shown previously (3) that Cd-rich phosphorus fertilizers, which were used in Finland in 1975-1981, caused an increase in the cadmium content of Finnish agricultural soils in general. Sewage sludge can be a source of all heavy metals (chromium, cadmium and lead). The amelioration may have caused on average higher accumulation of heavy metals in the sugar- beet fields than in other cultivated soils which have not required such intensive conditioning as the sugarbeet fields.

The aim of the present study was to elucidate the heavy metal (Cd, Cr and Pb) status of Finnish sugarbeet fields and sugarbeet in 1993 and to show the degree of contamination of the sugarbeet soil and crop by heavy metals. Special attention has been focused on the impact of soil pH on the availability of heavy metals to sugarbeet.

MATERIALS AND METHODS

A heavy metal study on the Finnish sugarbeet and sugarbeet fields was carried out in 1993. The material was collected from 152 trial farms representing the entire sugarbeet cultivation area in Finland and accounting to 3.6% of all sugarbeet growing farms in Finland. The locations of the farms are presented in Figure 1.

Plant Samples

Towards the end of the growing period, the beet and top yield of the trial fields were recorded on 20 September. The growing period ends in Finland usually in mid-October. The samples consisted of 20 randomly collected beets. The beets were topped manually with a sugarbeet knife. The tops were weighed on the field with a spring balance. A subsample of 1 kg consisting of tops was taken to the laboratory. The unwashed top samples were dried at 105°C, chopped and ground to pass a 2-mm sieve.

The sample beets were washed and weighed at the tare house of Salo sugarbeet factory. At sampling time, the density of the plant stand was determined for the calculation of the beet and top yields for each field. The deep-frozen brei samples (about 100 g) of the beets were analyzed in the laboratory of the Sugar Beet Research Centre for sugar and impurities. The dry matter content of beets was determined from the deep frozen brei samples, which were dried at 105°C. The plant samples were stored in plastic bags.

The dried top and beet samples were analyzed for heavy metals (Cd, Cr and Pb) at the Agricultural Research Centre in Jokioinen. For the determinations, a 5g sample was ashed overnight in a silica crucible at 450°C. The ash was dissolved in 10 ml of 3 N HCl and filtered into 50 ml volumetric flask. Filter paper with residue was ashed at 600°C, treated with HClO₄ and HF, dissolved in HCl and combined with main solution. The measurements were made by flameless atomic absorption spectrophotometry.

Soil Samples

The soil samples were taken from the plough layer (0-25 cm). Each sample consisted of 20 subsamples which were taken from near the beet sample. The samples were air-dried for analysis and the dried samples were passed through a 2-mm sieve. Soil classification was made by visual inspection of the samples. All soil samples were made organic matter content (O.M.), and a soil was classified as organic soil if the O.M. content was over 40% and as gyttja if O.M. was over 6% in sea sediment soil.

Bulk density was determined by weighing a 25 ml quantity of air-dried and ground soil. Soil pH(H₂O) was measured from a soil:water (1:2.5) suspension after letting the suspension settle overnight. The measurement was made conventionally with a pH meter from this suspension after stirring.

Phosphorus, potassium, sodium and magnesium were extracted from soils using a 0.5 N ammonium acetate 0.5 N acetic acid (AAAc) solution (pH 4.65), the extraction ratio being in a 1:10 volume ratio, the extraction time 1 h, and the shaking speed 27 rpm (19). P was measured from the soil extracts colorimetrically using the Mo blue method. K, Mg and Na were measured with an inductively coupled plasma emission spectrometer (ICP). The concentrations are given as milligrams per liter of soil.

Cadmium, chromium and lead were extracted from the soil using a 0.5 N ammonium acetate 0.5 N acetic acid 0.02 N Na₂EDTA (AAAc-EDTA) solution (5). The extraction time and extraction ratio were the same as in the AAAc extraction. Cd and Pb concentrations were measured by atomic absorption spectrophotometry using an air-acetylene flame. The concentration of Cr was determined with an ICP.

RESULTS AND DISCUSSION

Soil Types, Acidity and Macronutrients

Classification of the soils was made according to Aaltonen et al. (1). Distribution of the soils into different soil types is presented in Figure 2.

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The mean $\text{pH}(\text{H}_2\text{O})$ of the soils was 6.76 (Table 1). The fields of the trial farms were quite well limed as seen from the fairly high pH value. The trial farms were slightly better than the other farms as the pH value is concerned; because the $\text{pH}(\text{H}_2\text{O})$ was as low as 6.46 in 1990 in sugarbeet soils in general (11). In the cultivation area where the trial farms were located, the pH value was only 5.92 under other crops in 1987 (3).

The macronutrient status of the trial fields is presented in Table 1. The phosphorus status was better than in sugarbeet fields on average and much better than in the other fields of the same cultivation area. Potassium, sodium and magnesium status in the trial fields was quite similar to the figures presented by Pelo (11) and Erviö et al. (3).

Cr, Cd and Pb in Soil

The chromium, cadmium and lead concentrations in the soil of the trial fields are presented in Figure 3. The mean chromium, cadmium and lead concentrations were 0.31, 0.10 and 2.65 mg/l soil respectively. The figures are almost identical to the figures presented by Erviö et al. (3) for the same cultivation area. In that survey, the fields were not sugarbeet fields. In a survey by Sippola and Tares (16), a mean chromium concentration of 0.28 mg/l and 2.96 mg/l for lead were observed in non-sugarbeet fields in Finland in the sugarbeet growing region.

It is not easy to make a comparison of the extractable heavy metal levels of the soils between Finland and other countries since AAAC-EDTA is not a commonly used extraction solution for soil in the world.

However, Sillanpää (15) has reported on the AAAC-EDTA-extractable Cd levels in the agricultural soils of 30 countries under a FAO project. According to him, the national mean of AAAC-EDTA-extractable Cd concentration in Finnish soils in the mid-1970's was 0.11 mg/l soil. This was equally low as in the developing countries, in general and lower than the corresponding soil Cd concentrations in most of the developed countries in his study.

According to Sillanpää (15), the mean AAAC-EDTA-extractable Pb concentration of Finnish cultivated soils, 2.5 mg/l soil, in the mid-1970's, was distinctly lower than the national mean values of the other European countries compared: Malta 34, Belgium 13, Italy 12 and Hungary 6 mg/l soil.

Cr, Cd and Pb in Tops and Roots

Figure 4 shows the chromium, cadmium and lead concentrations in tops at harvest. The mean chromium, cadmium and lead concentrations in dry matter were 1.55, 0.472 and 6.74 mg/kg, respectively.

Figure 5 shows the chromium, cadmium and lead concentrations in roots at harvest. The mean chromium, cadmium and lead concentrations in dry matter were 1.83, 0.066 and 0.68 mg/kg, respectively.

The figures show that heavy metals concentrate in leaves rather than in roots. Lead concentration in the leaves was about 10 times, and that of cadmium 7 times, higher than in the roots. With respect to chromium, its concentration was almost equal in tops and roots.

It is not easy to make a comparison of heavy metal levels in beets between Finland and other countries since information is scarcely available. Then there is the uncertainty of the sample preparation for analysis - whether the sample has been washed or not. This has a major effect on the lead concentration of plant material, as pointed out by Kloke and Riebartsch (9), and on the lead concentration of the sugarbeet leaves (10)). In our study, the sugarbeet leaves were not washed before analysis and the beets were washed in the tare house of Salo sugarbeet factory. It is quite obvious that washing in a tare house is not as efficient as in the laboratory. Soil contamination is quite obvious when washing is done in a tare house.

In the following, figures found in the literature are presented in a descending order. The time factor can cause an error on the concentrations. Therefore the comparison must be done carefully:

Concentration in tops mg/kg dry matter

	Cr		Cd		Pb
Czech Rep. (12)	2.222	Present study	0.472	USA (10)	106
Present study	1.55	Germany (8)	0.41	USA (10)	60
Germany (14)	0.5	Netherl. (20)	0.31	Germany (8)	52
		Germany (8)	0.29	USA (10)	44
		Germany (14)	0.225	Germany (17)	11-29
		Czech Rep. (12)	0.194	Germany (8)	9.5
				Present study	6.74
				Netherl. (20)	3.6
				Czech Rep. (12)	3.332
				Germany (14)	0.625

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Concentration in roots mg/kg dry matter

Cr		Cd		Pb	
France (2)	12.3	France (2)	0.44	Germany (17)	1.3-9.6
Present study	1.83	Germany (8)	0.2	Germany (8)	6.3
Czech Rep. (12)	1.664	Germany (8)	0.15	USA (10)	2.3
Germany (14)	0.25	Czech Rep. (12)	0.082	Germany (8)	2.0
		France (2)	0.07	Czech Rep. (12)	1.664
		Present study	0.066	Present study	0.68
		Germany (14)	0.0625	USA (10)	0.4
		France (2)	<0.05	Germany (14)	0.275
				USA (10)	0.2

The cadmium level in the roots is quite low in Finland, and safe for the plant and for animals if the roots are used in animal feed (Table 2). Inversely, the cadmium level in tops is fairly high in Finland but safe both for the beet and for the animals.

The chromium levels in the roots and in the tops are moderate in Finland. They do not exceed the toxicity levels for plants and animals (Table 2).

The concentration of lead in the roots is very low in Finland. It is safe both for the plant and for the animals. On average, the lead content in the tops is moderate, but in individual cases it can exceed the toxicity limits for plants and animals (Figure 4 and Table 2).

Correlation between Heavy Metal Concentrations in Soil and in Plants

As seen from Figure 6, there does not exist any correlation between the heavy metal concentrations in the soil and in the roots.

Figure 7 also shows that there does not exist any correlation between lead and chromium concentrations in the soil and in the tops. Inversely, cadmium content in the tops significantly correlated with the cadmium concentration in the soil. The cadmium amount in the soil was dependent on the pH of the soil as seen from Figure 8. This has been pointed out by many investigators.

The correlation between heavy metals in the soil and in the plant was rather weak because there were too many unknown factors e.g. heavy metal deposits from air and soil contamination of the samples (18).

Correlation between Heavy Metal Concentration in Roots and Tops

As seen from Figure 9, only the cadmium content of the tops correlated significantly with that of the roots. This shows the pathway of cadmium from soil through roots to the tops.

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Table 1. Acidity, macro nutrient concentrations and bulk density in soil samples (n=149)

	Min	Max	Mean	(Pelo) (1991)	(Erviö) (1990)
pH, (H ₂ O)	5.48	7.65	6.76	6.46	5.92
P, mg/l soil	11	192	55	35	13
K, mg/l soil	23	445	158	186	170
Na, mg/l soil	15	104	48	55	-
Mg, mg/l soil	27	684	201	223	248
Bulk density	0.52	1.39	1.08	-	-

Table 2. Critical concentrations of heavy metals in plants and in animal diets. Higher levels are toxic (Sauerbeck 1982).

	Plants	Animals
	mg/kg DM	mg/kg DM
Cadmium	5-10	0.5-1
Chromium	1-2	50-3000
Lead	10-20	10-30

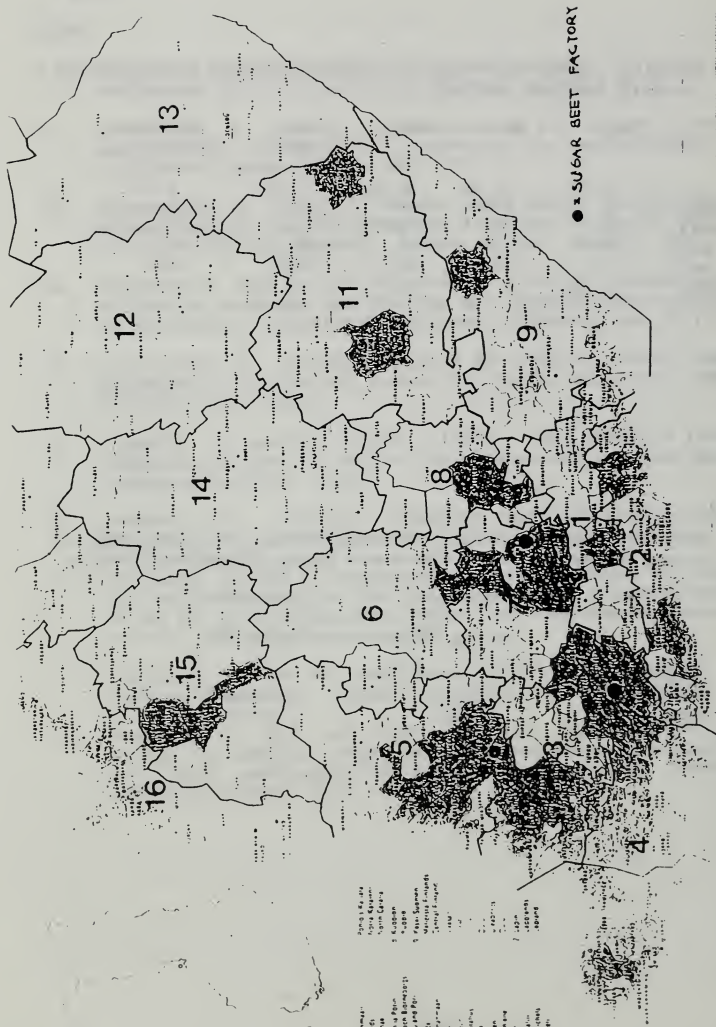


Figure 1. Soil and plant sampling sites (sites darkened).

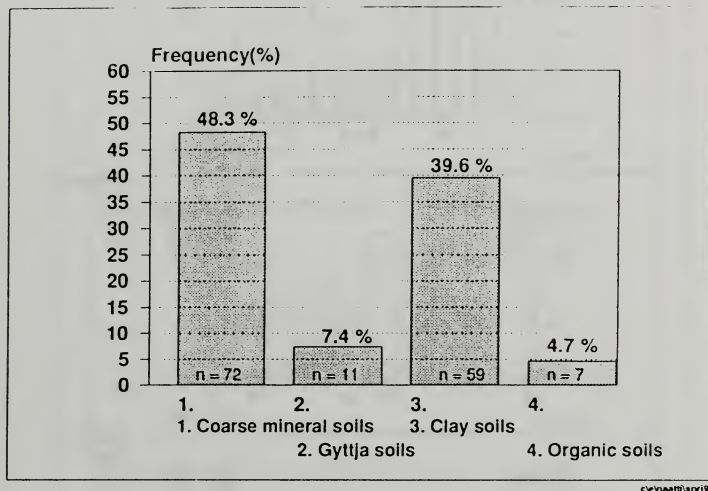


Figure 2. Frequency distribution of soil types.

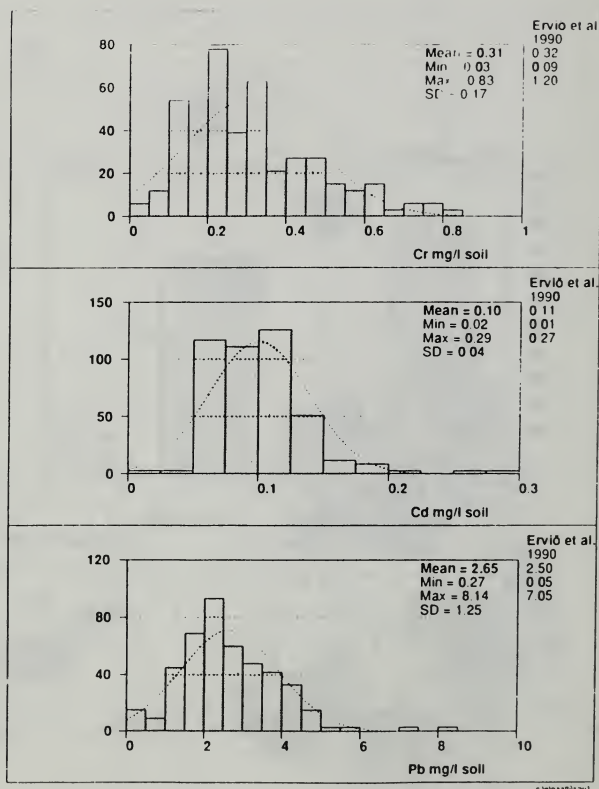


Figure 3. Distribution of soluble lead, cadmium and chromium contents in soil.

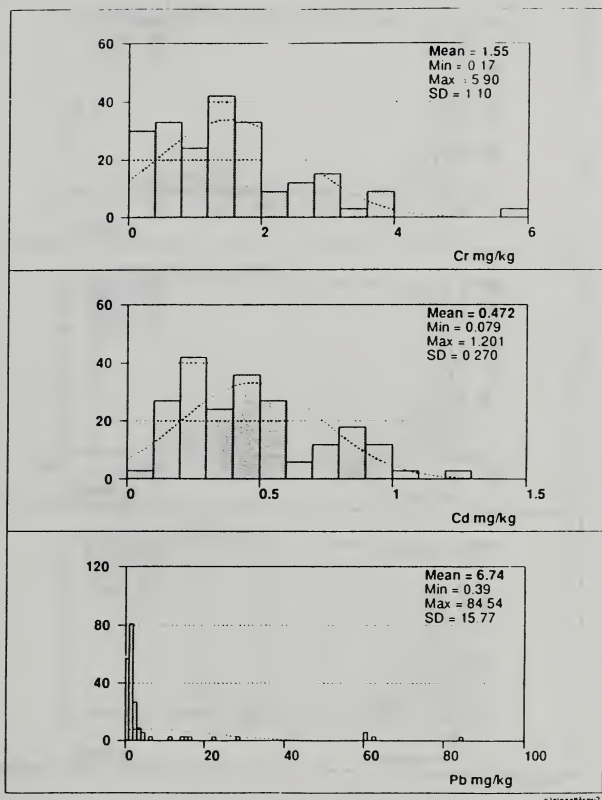


Figure 4. Concentrations of lead, cadmium and chromium in tops (dry matter).

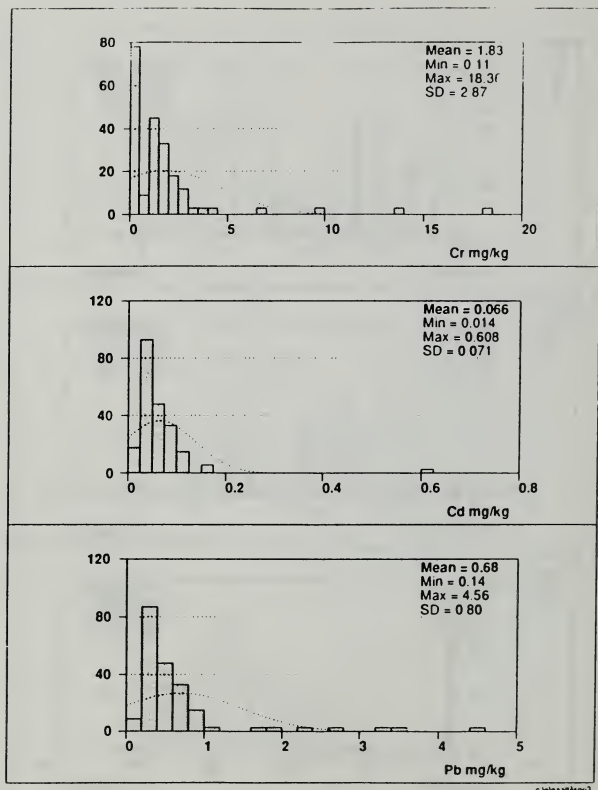


Figure 5. Concentrations of lead, cadmium and chromium in roots (dry matter).

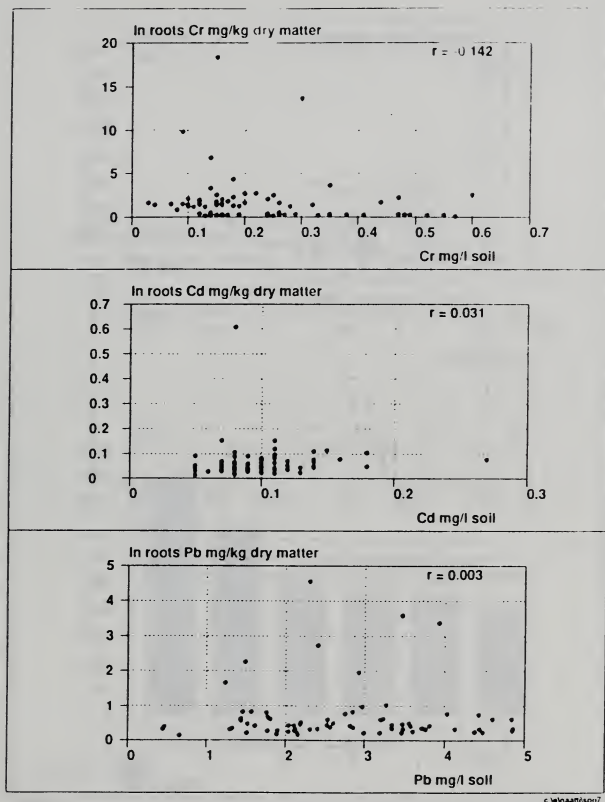


Figure 6. Correlation between lead, cadmium and chromium concentrations in soil and in roots.

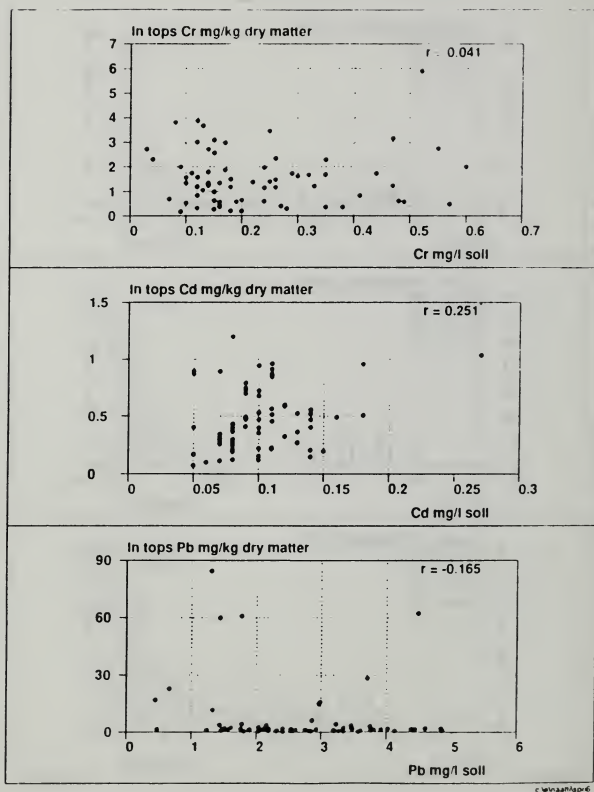


Figure 7. Correlation between lead, cadmium and chromium concentrations in soil and in tops.

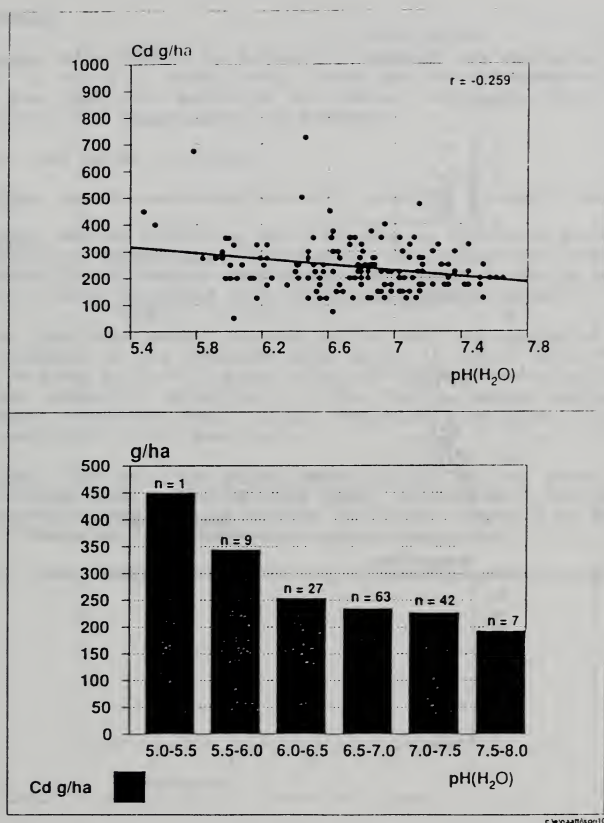


Figure 8. Correlation between pH and cadmium amount in plough layer.

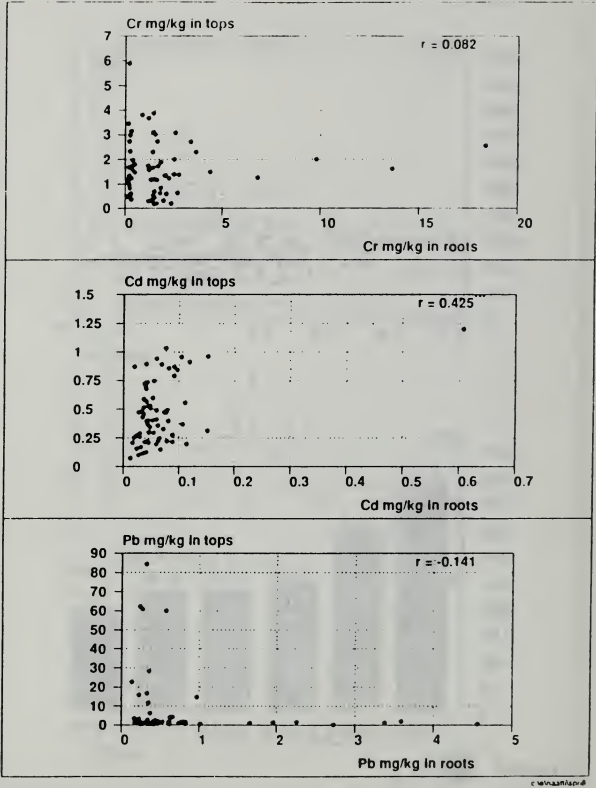


Figure 9. Correlation between lead, cadmium and chromium concentrations in roots and in tops.

DISCUSSION

Question: With regard to levels of cadmium and chromium levels in foods: in the U.S. these days, these are now elements of concern. Have you had any emphasis on these compounds from the food administration departments in Finland?

Erjale: Not to my knowledge.

Question: You'll certainly be well prepared if enquiries arise.

Question: You've collected a lot of data on levels of microelements in different countries. Presumably the analytical methods were different in different countries. I wonder if it is correct to compare levels measured with different methodologies?

Erjale: The heavy metal content in the soil was compared using the same methods in our studies with those in other countries. The data is from an U.N.-F.A.O. study of thirty countries done by a Finnish research scientist. The heavy metal analyses from different countries are, I think comparable because they have all been analyzed in the same way.

Question: You said the heavy metal levels in the roots was very low, almost zero - that's very good, of course. But still, the concentration per kilo of product is higher compared to per kilo of soil - there is some concentration by the plant.

Erjale: Yes, some regulations will probably be developed.

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THE OPTIMUM USE OF TAILS FROM BEET PROCESSING

Nicholas W. Broughton, David Sargent, Trevor C. Theobald and
F. Gaughan

British Sugar plc, Colney, Norwich, United Kingdom

ABSTRACT

This study has characterised the composition of various particle size fractions of tails from two British Sugar factories to provide data for decisions on what is economically optimal to do with this particulate sugarbeet material.

All fractions are of a much lower sucrose concentration and higher invert sugar concentration than cossettes. Sucrose concentration is directly proportional to particle size and its lower concentration compared to beet is likely to be associated with leaching in the transport system.

The purified juice derived from these fractions is of lower purity and higher colour than the equivalent juice from cossettes. The purity of the juice is directly proportional to the particle size.

In general, the purity of the purified juice from the fractions smaller than 5mm is lower than that of molasses. Processing of tails by extraction will therefore lead to a lower overall sucrose recovery. On the other hand, the inclusion of tails in animal feed reduces its quality.

The decision on how best to process tails is complex. It is impossible to define a universally acceptable procedure. This study identifies key aspects of the chemistry of tails and how they perform in processing, as part of the process of making an informed decision.

INTRODUCTION

It is often understood that sugarbeet tails are particulate, organic sugarbeet material in the size range 1-10mm arising from the transportation and cleaning of beets. Available data (1,2) suggest, however, that in many countries material considerably larger than this may also be classified as tails.

This material does not come exclusively from the tail of the beet root, despite the name. It comprises fragments broken from many parts of the root, usually from its surface.

It is widely reported (1,3,4) that the average proportion of tails is 1-3% on beet, containing 10-14% sucrose (3,5,6). These figures can vary substantially, depending on climate, geographical area and systems for beet lifting, transportation and factory handling (1,3,6). At a factory slicing 15,000 tpd and operating a 80 day campaign, the tails can thus contain 1,200-5,000 tonnes of sugar.

Studies in Poland (7) have shown that the concentration of sucrose is well correlated with fragment size: a pol value of 5.2% apparent sucrose has been determined for material with a diameter of less than 6mm (1). Presumably, for smaller particles, the greater surface area to volume ratio results in more leaching of sugar from damaged cells into the transport water. Based upon such information, tails material is often treated as waste.

Two key studies (1,2) have identified 3 ways in which sugarbeet tails can be utilised if they are not to be dumped:

- return the untreated material directly to pressed pulp for processing as animal feed;
- co-process the tails, either sliced or unsliced, with the beet cossettes by conventional extraction;
- prepare and extract the pieces separately in a special apparatus.

To these must be added the option to feed the separated material directly to the pulp presses, recover part of the sucrose and pass the remaining material forward for drying as animal feed.

The choice of which of these to exercise depends upon a number of factors and is often determined more by available plant than on economic considerations.

This study has looked at the cases of two British Sugar factories, each with different systems of screening to remove tails from transport water and of subsequent processing. It seeks to investigate the most cost-effective option for each factory by understanding how the material would behave in the factory diffusion and purification processes and in animal feed.

METHODS AND METHODS

- i) Practical studies have been carried out based upon beet materials collected from two British Sugar factories. During campaign 1993/94, samples of that material conforming to each factory's definition of the tails fraction were collected and manually separated into further discrete fractions on a series of Endecott laboratory sieves. To obtain sufficient beet material for testing required sampling and fractionation over a prolonged period. Each fraction was then thoroughly mixed before being divided into portions prior to storage. The material was immediately frozen on site for processing at a later date. Cossette material from each factory was collected and frozen at the same time, to provide comparative data.

At each factory, beets pass from a flat storage pad into fluming systems which incorporate stone and weed catchers. From there, they pass over dewatering screens either to beet washers or directly to process. That material passing through the dewatering screens both before and, if appropriate, after the beet washers constitutes the tails fraction.

At factory A, the beets are passed for dewatering over an 8mm wedge wire screen. The underflow, which contains the tails fraction, is then passed for tails recovery over a 1mm wedge wire screen, with the overs being further pumped over a vibrating parabolic screen. The tails fraction is sent to the driers with pressed pulp.

At factory B, the system is slightly different and the beets are passed over a Don Valley screen with 25 x 10mm slots. The underflow passes either to a tails drag system or over a 1mm Roberg screen, then, with further transport water over a dewatering screen. Material from the tails drag goes to the cossette belt for diffusion whilst that over the Roberg screen goes via the final dewatering screen to the driers.

Thus, in our study, all tails material was taken at the final point of its separation from transport water. In some other studies in the literature (5,7), it may be that the tails were collected from earlier stages of transportation and handling.

- ii) Each fraction was subjected to a laboratory batch diffusion consisting of four battery cells. 550g of beet material were introduced into each cell with 450 cm³ of pre-heated distilled water. The battery was maintained at 72°C and diffusion was allowed to proceed for 100 minutes.

At the end of this period, the cells were drained through a nylon bolting cloth with a 200 micrometre mesh and the wet pulp pressed in a laboratory hydraulic press under conditions chosen to give a pressed pulp dry matter comparable to that of the factory.

The press juice and the diffusion juice were combined for further processing.

- iii) Laboratory batch first carbonatation was carried out according to a procedure developed within British Sugar Research and Development to give comparable properties to the factory juices. A sample of 800cm³ of juice from each fraction underwent this laboratory batch carbonatation.

Because of the wide range of juice Brix (2-10.4Bx) derived from the diffusion phase, milk of lime (M.O.L.) addition based upon Brix would have resulted in vastly different added amounts, with the effect of producing variable first carbonatation juice quality. As the key objective of carbonatation is the removal from juice of the impurities, it was considered more logical to base the addition of M.O.L. on the concentrations of impurities in the juices. As these were all similar, both within the sample sets and to that routinely measured in the factory, M.O.L. addition was set at a fixed quantity equivalent to 1.2% CaO on beet.

The juice was rapidly heated to 85°C during which time the factory Wilfley slurry (≈7% on juice) was added. Once the juice was at temperature, the milk of lime was added over 8½ minutes, with simultaneous gassing with carbon dioxide, commencing at pH 11. On completion of M.O.L. addition, gassing was continued for a further 1½ minutes down to pH 11.2. The total time was 10 minutes.

The filtration and settling characteristics of this juice were then tested using standard British Sugar procedures. Filtration time refers to the length of time, in seconds, for a set volume of carbonatated juice to dewater, under vacuum, through an 8 micrometre filter under standard conditions. Its value gives an indication of the dewatering characteristics of the juice/mud on a rotary vacuum filter. Settling time is the length of time, in seconds, for the floc in a set volume of carbonatated juice to settle between 2 points on a standard measuring cylinder. This value will equate to juice performance in the factory clarifiers.

- iv) The juice was left to settle for 45 minutes, maintaining the temperature at 85°C. The supernatant was decanted and further

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heated to 90°C. The juice was then gassed with carbon dioxide to pH 9.2 over a 5-6 minute period. The juice was filtered through an 8 micrometre acetate filter before rapid freezing awaiting analysis.

- v) Samples of each 2nd carbonatated juice to which 200ppm SO₂ as ammonium bisulphite had been added were placed in a Carius tube. This was immersed in an oil bath at 135°C for 10 minutes, after which the tube was rapidly air cooled.

All particle and juice analyses were carried out using standard methods adopted in British Sugar. Sucrose was measured both by HPLC and polarimeter and invert sugar by HPLC and an optical method, based upon the reduction under alkaline conditions of triphenyl tetrazolium chloride.

RESULTS AND DISCUSSION

The data in Table 1 compare the relative proportions of the various size fractions from each factory. The Table also shows that the quantity of tails at both factories was in the range 1.5-2% on beet. The spot average for British Sugar factories over campaign 1993/94 was in the range 0.5-2%. The proportions of each size fraction were very similar between the two factories, with about 30-35% of the material finer than 4.75mm and about 60% finer than 6.7mm.

The sucrose and invert sugar concentrations of each fraction are given in Table 2. The invert sugar concentrations are expressed per 100g apparent sucrose. In comparison to cossettes collected at the same time, the sugar contents of the tails were much lower whilst the invert sugar concentrations were much higher.

At factory B, the maximum sucrose concentration in any of the tails fractions was 5.7% w/w. As the particle size decreased, so did the sucrose concentration. A similar pattern is seen for factory A, except that the sucrose concentration of the largest fraction was significantly higher at 9.4% w/w. This may be a result of a larger average particle size in this fraction at factory A, but no data are available on the particle size distribution of this largest fraction at each factory.

The sucrose concentrations of all of the tails samples were very much lower than those reported for tails in the literature (3,5,6). In this study, the material was sampled after the final dewatering screen. Loserth, in an earlier study (8), concluded that substantial losses of sucrose occur during tails fluming and washing because their mass, in comparison to the beet mass, has more

surface area, facilitating sugar leaching into the transport water, in particular from open cells. Thus, whilst the original mean sucrose concentration of the tails might be of the same order as in the taproot, it is highly unlikely that this will remain so once the material has passed along the beet transport and separation line. This would explain our observed correlation between particle size and sucrose concentration.

Moreover, it is well-known that the sugar content is lower towards the surface of the root, which is where much tails material originates. This too must contribute to the difference in sugar contents of tails and cossettes.

It is of interest to note also that, in her review on the processing of tails material, Tomaszewska (1) considers that tails fragments of less than 6mm diameter, which she found to have 5.2% apparent sucrose by polarisation, should be classed as impurities rather than processable waste. Her remedy was to remove this material and pass it directly to beet pulp as fodder.

The invert sugar concentrations of the fragments > 2.8mm were generally within the range 10-30 g/100S. The smallest particles (< 2.8mm) had a much higher invert sugar concentration, 60-80 g/100S.

There does appear to have been some degree of deterioration of the beet material in general prior to analysis as the invert concentration of the cossettes was rather high. It is not known whether all of this deterioration had occurred before sampling or whether some could have occurred during freezing and storage.

The higher invert sugar concentration in beet tails compared to cossettes is likely to be the result of a number of factors. Recent studies within British Sugar have shown that the invertase activity in beet tails is up to 10 times higher than that in cossettes. In addition, the large surface area of these particles would be expected to lead to a high adherence of transport water, which would contribute additional invert sugar.

The data in Table 3 show that a raw juice of only 9-10Bx was obtained from cossettes of about 17% sucrose. This was replicated pro-rata for all of the other fractions sampled. Thus, the juices for the tails fractions were in the range 2-5Bx. Two factors contributed to these low values compared with factory raw juice: first, use of a batch battery diffusion system, which was necessary to cope with the small amounts of the finest fractions; second, the impossibility of achieving steady state with such small amounts. To allow results to be comparable, the smallest amounts fixed the extraction procedures for all samples. The raw juices were within the range pH 5.9-6.5, with the tails fractions having figures at

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the lower end of this range. This may be related to adhering transport water.

True juice purities were also measured, as recorded in Table 3, based on sucrose by HPLC. There is grave doubt about the interpretation which can be placed on any measurements of purities of raw juices, because of interference from clay and soil solids. The data are presented only to show some major trends which are unlikely to have been affected by these factors.

The raw juice from cossettes, at 86.3-88.6% true purity, was similar to that which would be expected in the factory. As the particle size decreased, so did the raw juice apparent purity. This is likely to reflect the lower sucrose concentration. Hildebrandt (9) and Schonburg (10) in independent studies found that the purity of raw juice from tails was similar to that from cossettes. Although it is not clear, the material used in their studies had probably been collected before the transport system and was, therefore, of a higher sucrose concentration.

The properties of the 1st and 2nd carbonatation juices are given in Table 4. The settling times of the first carbonatation juices from tails were all lower than those from cossettes, which would suggest that, at clarification, there would be an improved settling of the lime mud. There is no obvious explanation for these lower values, although they may be related to lower juice viscosities in line with the lower Brix and sucrose values. Juice filtration times of all fractions were similar and this would suggest that there would be no penalty in terms of throughput on the rotary vacuum filters by processing the tails.

The apparent purities of the 2nd carbonatation juices are also given in Table 4. In calculating the purity of the 2nd carbonatation juice, a deduction has been made for the sucrose entering the system with both the Wilfley slurry and the milk of lime. In a normal factory scenario, this would not be necessary owing to these compounds being at constant and low amounts compared to the sucrose in the juice. In the tails fractions processed here, the effect of not taking this into account would be to increase the purities of all fractions, but in particular those of the smaller size fractions.

At about 93% apparent purity, the second carbonatation juice from cossettes was typical of that juice in British Sugar factories. The purities of the juices from the tails fractions were all lower than this, and reduced as the original tails particle size reduced, as had the raw juice purities.

The second carbonatation juices from the very finest tails fractions were below 50% apparent purity, especially at factory B.

At larger particle sizes, the purities rose towards or exceeded typical molasses purities. This is shown in Figure 1 for factory A and Figures 2 and 3 for factory B. Included for comparison is the line for mean molasses purity at each factory for the 1993/94 campaign.

Included in Table 4 are the data obtained from heating of the sulphitated second carbonatation juices at 135°C for 10 minutes in a miniature autoclave. Whilst the colour of the juice derived from the cosettes was slightly higher than that of the equivalent thin juice in the factory, the colours of the juices from all tails fractions were between 5 and 10 times that from cosettes. Although the pattern is not strictly followed, there does appear to be a relationship between particle size and colour (although, of course, this is not one of direct cause and effect). This in turn confirms the relationship between raw juice invert sugar concentration and evaporated juice colour that has been demonstrated by other workers (11,12,13). The effect of adding the tails fractions back into the process for diffusion will therefore be to increase the colour of the juice and ultimately that of the white sugar without additional corrective measures.

These results are shown in Figure 4, which plots colour of each heat-treated juice against the invert sugar content of the corresponding raw juice.

ANIMAL FEED CONSIDERATIONS

In trying to determine the optimum method of processing tails, it is also important to consider the effect that their inclusion may have on the quality of the animal feed product. Relative to the quantity of dry matter in pressed pulp, the return of all tails to pressed pulp may represent 3% additions of solids, as simple calculations show. Moreover, where tails include adverse nutritional components such as ash at concentrations on solids several-fold greater than in pressed pulp, the effect on these specific components in the final product can be much greater.

British Sugar currently processes 85% of its exhausted pulp through to a molassed sugarbeet feed. Under the U.K. Fertiliser and Feedingstuffs Regulations 1988, this product is classified as a straight feedstuff, for which key analytical parameters are given in Table 5.

Table 5 also includes values for these parameters (expressed on dry solids) determined for all the tails fractions. These values are compared with those typical for plain dried pulp.

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Of immediate concern when considering the inclusion of tails would be their effect on the gross energy of the product. It is clear that the gross energy of the tails is up to 20% lower than that of the conventional plain feed, with one value, that for the smallest fraction in the tails drag system of factory B, at 70% lower.

In general, the gross energy of the dried tails material is directly proportional to particle size. One of the major reasons for this is the much higher ash content of the tails fractions, which increases as particle size decreases. Thus, for the smallest fraction derived from the tails drag at factory B, the total ash on solids is 9 times that of the unmolassed product.

In factory B, much of the increase in ash appears to be associated with an increase in acid insoluble ash. Studies by Heinrich (6) have shown that grit is difficult to remove from the beet and that even, after extensive cyclone washing, up to 30% of the grit in the original material remains on the pulp.

All fractions have lower crude fibre contents than the standard product which, in combination with the factors described above, makes the tails fractions of significantly lower quality than the standard product.

It is likely, therefore, that addition of tails directly to feed will lower the overall quality. Some parameters, such as acid insoluble ash, may be much more adversely affected by this operation than others such as gross energy, because of the relatively greater differences in the concentrations of the former between tails solids and pressed pulp solids.

If tails are processed before return, especially by diffusion, the adverse impact on overall quality of feed is likely to be reduced, particularly because the ash content is likely to be reduced. But, as Section 3 shows, diffusion of tails leads to inversion, colour and purity issues to offset the benefit to feed quality.

PROCESSING OPTIONS

In considering the best options available for the treatment of tails, a number of factors has to be taken into account. It is not possible to make a universal statement on how they should be processed as the constraints of individual sites with regard to accounting method, equipment availability, effluent treatment capacity etc will all have an impact and fashion the final solution.

In the case of British Sugar and the examples discussed here, it is clear that diffusing those tails fractions giving a clarified juice

purity which is lower than that of the molasses currently produced will lead to an overall loss of sucrose from the bag. The financial impact of this will be offset to a small degree by the extra molasses produced.

If selective screening, either in single or double pass, is considered viable in order to return to diffusion only that material from which sucrose can be economically recovered, there remains a number of options on what to do with that material which does not go to diffusion. In British Sugar, these are:

- pass the material directly to the driers
- press the material before passing to driers
- discard it directly to transport water.

The first is likely to be the simplest option, incurring the minimum capital cost. The low dry substance of this material means, however, that it is likely to impose an additional evaporative demand on the driers which they may be unable to satisfy. Whether a factory can deal with this or accept the extra cost will be site specific.

Spoilage of the resultant dried product during storage through the growth of moulds may also be a problem, especially for the highly molassed product (14). This spoilage will arise in 2 main ways:

- a) short-term variability of tails material and feed rate, affecting the water load to the driers and so causing variation in product dry substance;
and
- b) non-uniform, often thicker, sections of the tails material, resulting in under-dried areas of product.

Problems with handling are also likely to arise as the pellets bond together (14). Under market conditions where ever-increasing pressures are being exerted to ensure that molassed sugarbeet feed handles at least as well as competitive compound feeds, this type of scenario must be avoided.

The second option seeks to overcome the problems associated with the first by pressing the material prior to passing it to the driers. It has already been accepted that the press water cannot be returned to diffusion supply owing to its lower purity. The tails therefore require a separate pressing with the press water then passing forward to effluent treatment. This will incur a major cost which will be only partly offset by the lower drying costs.

The final option of passing the material directly for effluent treatment incurs the costs of this treatment without any of the

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benefits of increased animal feed. Unless there is an extraordinary reason why this route should be considered, e.g. no drier capacity available, then this route is unlikely to be attractive.

The literature offers the opinion (1,15,16) that the most suitable route for dealing with beet "waste" is to process it using a separate processing line up to and including raw juice. This involves properly preparing the material for extraction. Rough cut slices are not recommended. Rather, it is suggested that the cells are mechanically opened by the use of a grating mill (17). This in part overcomes the differences in diffusion coefficients for cossettes and tails which are detailed by Vukov (18) and which would be a feature under the conditions of conventional diffusion. The material is then heat denatured in a short second stage before extracting the sucrose in cold water in a mixer and separating the pulp/juice by suction or centrifugation (19). Because of the size of the tails material and the fact that it has an open structure, its advocates believe that this type of process would be adequate and would have large savings in thermal energy. Clearly, more elaborate, but usually even more capital-intensive processes, could be proposed. This option has not been considered here as there is currently insufficient evidence of operation of this type of process in other plants to enable a reasoned judgement to be made on its merits.

British Sugar is now considering what changes, if any, should be made in the way each factory processes its tails. As the screening method of each site is different, this will affect the composition of the tails and hence each site will need to be evaluated independently. In addition, as the sucrose concentrations in all the fractions are low, the ability to extract the material will be significantly affected by the composition of the press pulp water return. The actual degree of sucrose extraction may therefore be different from that which we might expect based upon the extraction of cossettes.

CONCLUSIONS

The particulate beet material known as 'tails' is far from homogeneous. In general, its value either for processing through diffusion or for direct addition to animal feed falls off markedly the finer the particles. Moreover, even the larger particles, at their point of recovery from transport water, are of lower technological value than cossettes.

It is probably the immersion of these particles in transport water which accounts for these compositional differences, especially if it is considered that many of the smallest pieces were originally bigger pieces which have broken down over longer times in the

water. The fact that some of the pieces will have originated from near the surface of the beet must also be pertinent.

Our results indicate that only the coarser fractions do not adversely affect extraction. The high invertase activity of tails may also increase sugar losses if the tails are put through the extraction systems without efficient de-activation of their enzymes. Extra colour is also formed by processing tails.

Whether it is cost-effective to segregate the coarser particles depends in part on the ways in which the finer particles can then be processed.

Incorporation of all tails material, or just the finer material, in pressed pulp can adversely affect quality, storability and handling properties of the ultimate animal feed.

Given the existing capital investment in each factory, a detailed appraisal is needed to reach a decision on the optimum processing route for tails at each site. No universal solutions can be given, but this paper identifies key scientific factors which must be taken into account.

ACKNOWLEDGEMENTS

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Table 1 Relative Percentages of each Size Fraction
in the Tails Material from Two Factories.

Size Fraction	Factory A Proportion (% w/w)	Factory B Proportion (% w/w)
< 2.8mm	4.4	8.3
2.8-4.75mm	24.8	27.9
4.75-6.7mm	27.0	25.5
> 6.7mm	43.8	38.3
Tails (% on beet)	1.95	1.5

Table 2. Comparative Sucrose and Invert Sugar Concentrations for each Size Fraction.

Sample Source	Fraction	Apparent Sucrose (%w/w)	Invert Sugar (g/100S)
Factory A	Cosettes	17.3	1.5
	Tails < 2.8mm	1.8	59.9
	Tails 2.8-4.75mm	3.0	24.4
	Tails 4.75 - 6.7mm	4.8	19.2
	Tails > 6.7 mm	9.4	6.7
Factory B (Tails Drag)	Cosettes	17.4	1.9
	Tails < 2.8mm	1.0	77.5
	Tails 2.8-4.75mm	3.9	23.9
	Tails 4.75 - 6.7mm	4.8	11.8
	Tails > 6.7 mm	5.7	18.5
Factory B (Screens)	Cosettes	17.4	1.9
	Tails < 2.8mm	1.9	33.6
	Tails 2.8-4.75mm	2.6	30.9
	Tails 4.75 - 6.7mm	3.1	25.4
	Tails > 6.7 mm	5.2	24.1

Table 3 Comparative Raw Juice Quality for each Size Fraction

Sample Source	Fraction	pH	Bx	True Sucrose (g/100cm ³)	Invert (g/100S)	True Purity (%)
Factory A	Cossettes	6.5	9.5	8.7	3.0	88.6
	<2.8mm	6.0	2.5	1.0	73.1	41.1
	2.8-4.75mm	6.3	3.3	1.9	29.5	57.7
	4.75-6.7mm	6.1	4.2	2.8	18.4	66.3
	>6.7mm	6.2	4.6	3.1	21.8	66.6
Factory B (Tails Drag)	Cossettes	6.5	10.4	9.3	2.9	86.3
	<2.8mm	6.0	2.0	0.5	168.0	24.7
	2.8-4.75mm	6.1	3.1	1.6	44.9	50.4
	4.75-6.7mm	5.9	3.0	1.5	47.4	50.7
	>6.7mm	6.0	4.8	3.3	22.0	67.9
Factory B (Screens)	Cossettes	6.5	10.4	9.3	2.9	86.3
	<2.8mm	6.0	2.8	0.6	103.1	22.9
	2.8-4.75mm	5.9	3.2	1.2	61.0	38.0
	4.75-6.7mm	6.0	3.3	1.6	46.2	47.9
	>6.7mm	6.0	4.8	3.4	17.9	69.7

Table 4. Comparative 1st and 2nd Carbonatation Juice Qualities for each Size Fraction

Sample Source	Fraction	1st Carbonatation Juice		2nd Carbonatation Juice		Heated Thin Juice Colour (I.U.)
		Settling Time (secs)	Filtration Time (secs)	Bx	Apparent Purity (corr) (%)	
Factory A	Cossettes	56	52	10.1	92.9	3,300
	<2.8mm	32	71	3.7	48.2	30,900
	2.8-4.75mm	38	63	4.0	69.8	18,600
	4.75-6.7mm	32	67	5.1	76.0	13,800
	>6.7mm	39	77	5.6	64.6	15,200
Factory B (Tails Drag)	Cossettes	55	75	10.9	93.1	3,000
	<2.8mm	30	41	3.1	27.2	30,700
	2.8-4.75mm	26	89	4.1	52.7	22,500
	4.75-6.7mm	25	94	4.1	50.2	30,300
	>6.7mm	23	93	5.4	70.7	18,800
Factory B (Screens)	Cossettes	55	75	10.9	93.1	3,000
	<2.8mm	20	113	3.2	32.7	30,700
	2.8-4.75mm	18	84	3.7	44.7	33,500
	4.75-6.7mm	33	40	4.2	50.0	34,700
	>6.7mm	24	66	5.0	75.2	13,000

Table 5. A Comparison of the Animal Feed Properties of the Tails Fractions

Sample Source	Fraction	Analyses				
		Total Ash (%)	Acid Insol. Ash (%)	Crude Protein (%)	Crude Fibre (%)	Gross Energy (kj/kg)
Non-molassed Sugar Beet Pulp	-	8.1	3.5	8.8	18.6	16,500
Factory A	<2.8mm	12.9	4.6	9.4	10.5	14,100
	2.8-4.75mm	10.0	2.6	9.4	9.5	15,700
	4.75-6.7mm	8.9	1.7	9.6	9.9	15,900
	>6.7mm	7.5	1.4	8.8	9.1	16,100
Factory B Tails Drag	<2.8mm	72.8	54.9	3.6	4.9	4,500
	2.8-4.75mm	19.9	12.3	9.7	8.7	14,200
	4.75-6.7mm	14.4	8.8	10.3	8.9	15,000
	>6.7mm	12.1	7.3	8.9	7.0	15,300
Factory B Screens	<2.8mm	38.3	16.4	8.2	9.2	15,800
	2.8-4.75mm	26.0	8.7	10.2	11.0	13,200
	4.75-6.7mm	23.1	5.8	10.4	9.8	13,500
	>6.7mm	7.7	2.1	7.4	7.5	15,800

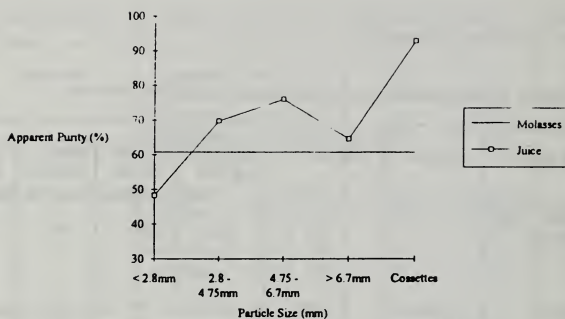


Figure 1. The Apparent Purity of 2nd Carbonatation Juice for the Tails Fractions from Factory A.

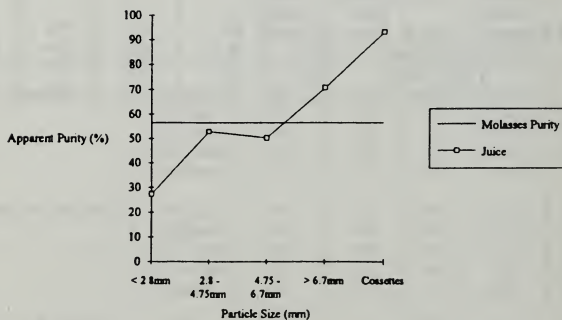


Figure 2 The Apparent Purity of 2nd Carbonatation Juice for the Tails Fractions from the Tails Drag, Factory B.

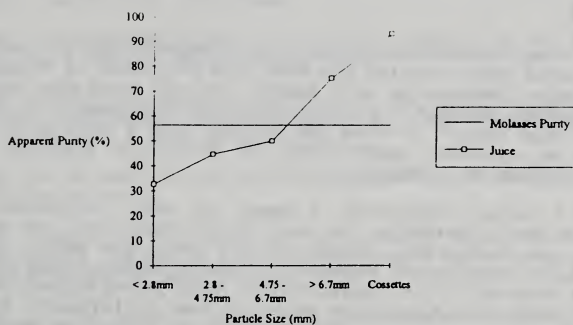


Figure 3. The Apparent Purity of 2nd Carbonatation Juice for the Tails Fractions from the Tails Screen, Factory B

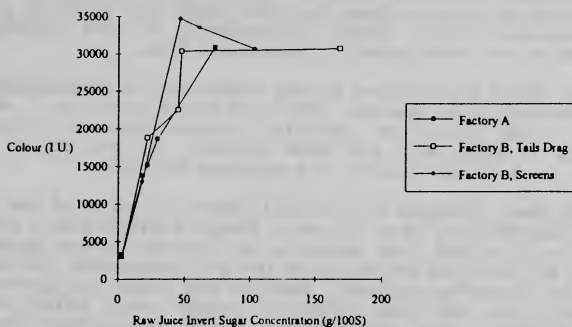


Figure 4 The Effect of Raw Juice Invert Sugar Concentration on the Colour of Heat Treated Thin Juice

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DISCUSSION

Question: Congratulations on your fine presentation, with excellent data. I would like to point out that the influence of fragments on the extraction process is well known. We all know the importance of the "Swedish number". I agree with your statement, "Prevention is better than cure," because the "tails" are a type of fragment. Also, in my opinion, extraction is difficult because of the clogging created by fragments.

Theobald: I agree completely. I have, in fact, mentioned in the paper that the literature is very clear that, as far as tails fragments are concerned, there is not only the point that you mention, that clogging of screens can occur because this material, especially the very fine, could travel with juice rather than with pulp, but also that the tails material has a different diffusion coefficient (this is documented) to that of cossettes material.

Question: How did you separate the tails from the flume water and other material in the flume system?

Theobald: The tails were already separated because they were actually gathered from the last dewatering screen, either the tails drag or the vibrating screen as shown in the slides. If you are also asking, how did we separate that from, perhaps, green material, that was removed separately. Another point that may be of interest to you is that we know that the mixture with green (tops) material will behave differently to the straight tails fragments as we have actually processed them.

Question: Would you comment on the contribution to suspended solids and to turbidity in general from the tails material. We've been working a lot lately on material present as, or arising from, turbidity in both beet and cane juices. Do you think the tails fraction is a major source of suspended solids?

Theobald: Yes. Because of the small physical size of the material, and the possibility that it could respond differently from normal material - it could, for example, go through to the carbonatation system - it could be responsible for problems under the conditions of high pH in carbonatation. Pectins and other polysaccharides be extracted from the beet material rather than being removed by carbonatation. It's another case where preventing formation is better than trying to cure problems later in process.

Question: Would you care to go into detail on the prevention procedures?

Theobald: Obviously, in British Sugar we have made significant investments in replacing the very clumsy and damaging mechanical

handling systems with the gentler flat pad systems. I don't have a current view on the next stage.

Question: In your autoclave experiment, were the pH's of your cossette juice and tails juice the same?

Theobald: The pH's were adjusted via the second carbonatation process and then sulfitated with ammonium bisulfite, to get a good representation of a true thin juice.

Question: So it may be the cation composition difference that is affecting the color?

Theobald: Possibly but surely the invert level has a large contribution to the color difference - there is a lot of evidence in the literature to that effect.

Question: In your report, the difference between composition of the tails and other parts of the beet is not great, but the decreasing size of the pieces affects the level of sucrose concentration, the purity, and so on. Will you explain.

Theobald: As far as the sucrose concentration is concerned, it is our belief at the moment that the tails material originally had a similar source concentration to that of the rest of the beet. But, because the tails particles are so small, and are passed around in the transport water system, then obviously you get considerable leaching. As to the differences in purity, a contributing factor may be the high invert sugar concentration measured in the tails fragments.

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THERMODYNAMIC ACTIVITY OF WATER AND SUCROSE AND THE STABILITY OF CRYSTALLINE SUGAR

Catherine Bressan and Mohamed Mathlouthi

Association Andrew VanHook and Laboratoire de Chimie Physique
Industrielle, Université de Reims-Champagne-Ardenne, Reims, France

ABSTRACT

Practical problems like caking and stickiness of crystalline sugar after drying and the curing of sugar lumps need to be solved with a minimum of theory. As each of the crystals is surrounded by a thin film of syrup, the thermodynamic and kinetic conditions for this film to reach saturation stability after recrystallization of sugar in excess are useful to define. Such conditions are dependent on water activity and sucrose activity in the film submitted to varied temperatures and water vapour pressures during storage.

After recall of water-sugar interactions in saturated solution and the relation giving water activity of sucrose solutions, taking into account the hydration number = 5, the authors summarize the work of the late Professor Andrew VanHook on the activity of sucrose in the film surrounding the crystal. The thermodynamic and kinetic aspects of crystal growth relevant to the treatment of the subject, stability of crystalline sugar, are recalled, mainly based on papers by Andy VanHook.

Experimental results of study of the curing of sugar lumps are reported. Interpretation of the evolution of moisture and water activity during the storage of the cubes is made. It is suggested that simultaneous crystallization and release of water are needed to obtain stability of the product. This requires that the heat of crystallization be used to evaporate the residual moisture. Preheating the sugar at about 60°C, the glass transition temperature of amorphous layer that might stick on the surface of the crystal, proves to be helpful in achieving a good curing at normal temperature and short time.

INTRODUCTION

The problems posed by the stability of sugar, its curing and preservation, of caking are generally approached as drying or storage at controlled relative humidity rather than crystallization

in the thin film surrounding the sugar crystal. Sugar technologists are not the only ones to think that crystalline sugar after processing and drying is a stable product; it is also the case for users, and the sellers who happen to place side by side very humid substances with sugar.

The practical problem of the stability of sugar during storage needs to be solved with the use of a minimum of theory. But one should choose a good theory. As concerns the stability of crystalline sugar or that of sugar cubes, it is most important is to be aware that each crystal is surrounded with a film of saturated or supersaturated solution. The concentration of this solution in sugar and water should be expressed in terms of activity rather than mass percentage. One should also take into account the kinetics and thermodynamic conditions of crystallization of sugar in the film of syrup around the crystal. These conditions are more important than the drying carried on to reach a certain moisture content.

Physico-chemical modifications which occur during the curing of crystalline sugar or sugar cubes are now studied from the point of view of the stability of water activity. Temperature is one of the parameters which may perturb the equilibrium of hydration. We will only deal with the curing of pure white sugar even though the presence of traces of impurity may influence the solubility and hydration of sugar.

Water vapour sorption isotherms are useful for the control of drying, storage and packaging of granulated sugar and sugar cubes. These isotherms can be interpreted at three levels: the thermodynamic, the structural and the technological levels. For crystalline sugar, the structural level is well documented in the numerous references on sucrose crystallization. This is not the case for food products containing sugar, which are treated according to the so-called "Food Polymer Approach" of Levine and Slade (7). Thermodynamic importance of water activity of sucrose solutions is evident from the treatment of sucrose as an ideal solute and the tentative application of Raoult's law to calculate sorption isotherms. However, decrease in water activity by sucrose molecules is much more complex than that of an ideal solute. On the technological level, we may find all aspects related to the interactions between the relative humidity of air and the saturated syrup surrounding the crystals during the drying process or the packaging and storage of sugar. This aspect is particularly difficult to master if amorphous sugar is formed. We intend to approach the effect of water activity on sugar stability using the three levels of understanding of the problem.

WATER-SUCROSE INTERACTIONS IN SOLUTION AND SOLID STATE:

Sucrose like other sugars was often studied in dilute aqueous solutions because it is a good model of a non-ideal solute which may be treated using the formalism of ideal solutions. However concentrated (saturated and supersaturated) solutions drew less attention from researchers. The most pertinent contribution in the field are due to Dunning (4) and VanHook (21) which were interested in sugar crystallization. Although one of the author's (Mathlouthi, 10) interest was focused on the study of the preponderance of sugar-sugar or water-sugar interactions in concentrated solutions, the approach adopted in this work takes particularly into account the water-sugar interactions in a saturated solution surrounding a crystal as a function of relative humidity, temperature and crystal size.

Water activity of concentrated sugar solution:

If sugar solution behaves as an ideal solution, water activity should be equal to the mole fraction of water:

$$A_w^{id} = N_w^{id} = \frac{55.51}{55.51 + m} \quad (1)$$

m being the molality of sucrose (mol/kg of H_2O). Because of the hydration, the activity of water is always lower than that of the ideal solution. The hydration water molecules (w) are withdrawn from the mole fraction, which gives:

$$A_w = \frac{55.51 - w}{55.51 - w + m} \quad (2)$$

where $w = nm$ and $n = w/m$ is the average hydration number for sucrose. Water activity of sugar solutions may be calculated (1) using equation 2 which takes into account the hydration water molecules. Hydration numbers of sucrose widely vary depending on the technique of measurement used. They were found to vary from 1 to 21 H_2O per sucrose molecule (2). It seems, however, reasonable to adopt a hydration number = 5, which fits either with calculated or with experimental A_w data, especially by Robinson and Stokes (11). If $n=5$, equation 2 becomes:

$$A_w = \frac{55.51 - 5m}{55.51 - 4m} \quad (3)$$

Application of equation 3 permitted calculation of A_w (1). Hydration number is represented as a function of sucrose concentration (see Figure 1). It may be noted that below a limit of concentration of 1.8 m or 38.1% dry matter, n remains constant. This limit corresponds to the preponderance in aqueous sugar solution of water-sucrose interactions. This hinge concentration was also found to correspond to the beginning of exponential increase in solution viscosity and interpreted (13,15) as a range of structural change in aqueous sucrose solution. Figure 2 shows the isotherm curve of sorption of water vapour of aqueous sucrose solutions. It may be observed that the change in slope of curve is situated around $A_w = 0.83$ which corresponds to saturation concentration at 25°C. This zone of A_w is essential to monitor the storage stability of crystalline sugar.

Hygroscopicity and water activity of crystalline sugar:

Although sucrose is one of the purest industrial products, it is nevertheless known that traces of impurities remain even in the best quality refined sugars. That is why the adsorption of water at the surface of the crystal greatly depends on the traces of impurities and it is almost impossible to find sugar free from moisture. Even if the air surrounding a sugar crystal is dried with silica gel and has its R.H. = 0, there is always a residual moisture content in sugar. The sigmoid curve of sorption of water vapour by sugar with moisture content = 0 for R.H. = 0 is just a theoretical result like the eutectic point of sucrose in water, only found from the intersection of cryoscopic and solubility curves and never determined experimentally (8). The water vapour sorption curve of crystalline white sugar is shown in Figure 3. It consists of a plateau with a constant low amount of moisture below $A_w = 0.83$ and an inflexion point at this A_w with an almost right angle. What is measured is the moisture of the film of syrup surrounding the crystal. In solid state, sucrose is essentially anhydrous. However, it is not stable like a crystal of quartz for example. It is permanently decomposed and recomposed at its surface owing to water, sugar and impurities found in the surrounding film. It was found, for example that water activity is decreased when the amount of impurity is increased (6). (See Figure 4).

Another parameter affecting water activity is the size of crystals. Indeed, experimental results showed that crystalline sugar with an average size of particle 150 μ m adsorbs more water than the sample

at 360 μm . The effect of crystal size was found even more important than that of temperature varied between 27 and 50°C. The effect of temperature is minimised after washing the crystals with ethanol saturated with sucrose. In all cases the inflexion takes place at around 0.83 Aw (6).

Amorphous sugar obtained by grinding or freeze-drying is more hygroscopic than the crystalline sugar. It adsorbs more water rapidly. This is due to a higher specific area on the one hand and to a probable modification of conformation around the glycosidic linkage on the other, which increases its reactivity towards water. The kinetics of water vapour adsorption by amorphous (freeze-dried) sugar are shown in Figure 5. After a rapid increase in moisture, there is a crystallization which is accompanied by a dehydration (9). The rapidity of water release from sugar after crystallization can not be explained only by a desorption in the surrounding atmosphere. It is certainly due to the heat of crystallization.

Activity of sucrose in the film surrounding the crystal:

The rapidity of crystallization of amorphous sugar may also be interpreted by the fact that amorphous ground or lyophilised sugar is in an activated state. On the one hand, the sugar molecules are well oriented to receive others around the microcrystals, and on the other, the growth of crystals does not need the energy barrier of water disassociation before incorporation. Indeed there is almost no syrup film surrounding the molecules of freshly milled or lyophilised sucrose as is the case for normal crystalline sugar. This film of syrup is at the origin of the instability of crystalline sugar and cubes during the storage in bulk or in a package. This instability is linked to the crystallization of sucrose in the film. The driving force of crystallization is the activity of sucrose with saturation taken as the standard state (20). More precisely, the driving force of the growth of sucrose crystals in a supersaturated solution surrounding the crystal is the difference of chemical potential between the solution and the crystal (or the saturated solution).

$$\begin{aligned}\mu - \mu_s &= RT \ln \frac{A}{A_s} = RT \ln(1 + \sigma) \\ &= RT\sigma \quad (\text{for } \sigma \leq 0.1)\end{aligned}\tag{4}$$

$$\sigma \text{ being supersaturation: } \sigma = \frac{A - A_s}{A_s}$$

A: solution activity
A_s: saturation activity
 μ : Chemical potential

Activity is a dimensionless number. It is related to concentration C by $A = \gamma C$ where γ is the activity coefficient.

From (4) we may obtain:

$$\begin{aligned}\mu - \mu_s &= RT \ln \frac{\gamma}{\gamma_s} \times \frac{C}{C_s} \\ \mu - \mu_s &= RT \ln \frac{C}{C_s} \equiv RT\sigma + RT \ln \gamma\end{aligned}\quad (5)$$

If saturated solution is taken as the standard state, $\gamma_s=1$. For γ close to 1, the approximation neglecting γ may be adopted to at least $\sigma = 0.1$. While s is dimensionless, its value will depend on the unit chosen for C . Depending on the concentration unit which may be expressed in mole fraction (X), molality (M) or volume concentration (% w/v), supersaturation $\sigma = (C/C_s) - 1$ has different values. VanHook (20) gives the numerical values of supersaturation for a solution with 70.2% dry matter (d.m.) concentration at 30°C, the saturation concentration being 68.18%. These values are respectively σ_m , σ_x and σ_v if concentration is expressed as M , X or C_v .

$$\begin{aligned}\sigma_m &= 0.10 \\ \sigma_x &= 0.089 \\ \sigma_v &= 0.040\end{aligned}$$

If supersaturation s is expressed as the ratio of weight concentrations:

$$\sigma_w = (70.20 / 68.18) = 1.030$$

Chemical potential may also be written differently depending on the unit of concentration:

$$\mu - \mu_s = RT \ln \frac{A}{A_s} = RT \ln \left(\frac{\gamma m}{\gamma_s m_s} \right) = RT \frac{f_x}{f_s} = RT \ln \frac{\alpha C}{\alpha_s C_s} \quad (6)$$

m , x and C are respectively molality, mole fraction and volume concentration.

Activity coefficients were not found to vary with temperature when concentration is expressed as m or x . The variation (see Figure 6) observed with volume concentration is only due to the effect of temperature on density (20). Thus, it may be noticed that sucrose activity as well as water activity are not affected by temperature.

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THERMODYNAMIC AND KINETIC ASPECTS OF CRYSTAL GROWTH:

VanHook proposed since 1944 that the activity of sucrose in supersaturated solution is the driving power of sucrose crystallization, which gives an expression of the rate of growth R_c :

$$\begin{aligned} R_c &= k (\mu - \mu_s) \\ &= k RT \ln \frac{A}{A_s} = k RT \ln (\sigma + 1) \end{aligned} \quad (7)$$

For low supersaturations ($\sigma < 0.1$), this gives:

$$R_c = k RT \sigma$$

Using the data from literature (Khukharenko, Smythe, Jackson), VanHook (19) calculated the rates of crystal growth and found discrepancies between the different authors on the one hand, and a marked effect of temperature on the other.

Effect of supersaturation:

The factor of potentiality of crystallization being the difference of activity or concentration between the considered solution and the saturated one, it is important to know with a maximum of accuracy the standard state defined by solubility. Numerous solubility tables are known, amid which Charles (3) data were adopted by ICUMSA. Solubility equilibrium may be approached from supersaturated or undersaturated side. The latter was found to give the most accurate results. Indeed recrystallizing sugar from a supersaturated solution always leaves a slightly supersaturated film around the crystal and leads to an equilibrium above $\sigma = 1.0$. The variation of solubility in function of temperature has been expressed by mathematical relations. Different authors (22,23) have noticed a discontinuity of sucrose solubility in function of temperature at 40°C. Although some sugar complexes like the hemipentahydrate were supposed to be at the origin of the discontinuity, it is more likely that this is due to an anomaly (16) in physical properties of liquid water which shows a minimum of specific heat at about 37 to 42°C. Smythe (14) obtained rates of growth with a maximum at about 40-50°C. VanHook (19) suggested that crystallization is a two-step mechanism: A step of mass transfer from solution to the crystal-solution interface and a step of dehydration-disassociation of hydration water and integration of sucrose molecules into the crystal. At high temperature, the energy of activation of diffusion is higher than that of integration whereas at lower temperatures the energy of activation of the

incorporation of molecules into the crystal (15 Kcal) becomes the limiting parameter compared to diffusion (5 Kcal) (see Figure 7). Moreover, growth rates were found linear in function of supersaturation except for low values of s where this linearity is not observed.

Effect of crystal surface:

The variation of crystal growth in terms of supersaturation was not observed by VanHook. Different authors observed this phenomenon especially Valcic (17) who has demonstrated that the differences in growth between low and high values of supersaturation are stumped if the number of dislocations is increased (see Figure 8). Crystallization in the thin film surrounding a crystal depends on the morphology of this crystal. The orientation of sucrose molecules and their binding by hydrogen bonds follows a selective development of the faces of the crystal. This aspect of sucrose crystal growth is exacerbated in presence of impurities like raffinose. The growth rate is different for a simple crystal in a mother solution frequently renewed, in an industrial pan with agitation, or in a sugar cube where the film of syrup surrounding the crystal is used, at the same time, as a liquid bridge between the crystals and to feed the growing crystal. These three cases may be represented as in Figure 9 by adaptation of scheme previously proposed by VanHook. The specific surface of the crystal being an important factor in growth rate, it seems evident that the larger the surface, the more rapid the growth. However, fine particles and amorphous sugar are dissolved even in supersaturated solution.

TECHNOLOGICAL ASPECTS:

It is well known that sugar drying, conveying and storing in silos is very often accompanied by dust production which presents a risk of explosion. The fine grain (below 200 μm) and the dust may also be risky as concerns the caking. Indeed, silo constructors recommend that the grain size of sugar stored in silos be higher than 200 μm . Dust is formed by breaking of crystals in the dryers, during transport, or by the fall against the walls of the silo. Fine grain also originates from a misconducting of first strike boilers or crystallizers. The granulometry is better controlled during the crystallization process and the formation of amorphous sugar may be avoided if drying is performed slowly at moderate temperatures.

Evolution of water activity:

As already mentioned, adsorption of water vapour by crystalline sugar is only rapid and risky for stability when the value of $A_w =$

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0.83 is reached. Temperature may favour the adsorption of water. However, we observed the evolution of a solution at 70°d.m. in function of temperature and found that A_w calculated from the ratio P / P_0 remains constant for temperature above 40°C (see Table 1). Moreover, it is observed during the first steps of curing of sugar cubes that a variation of moisture content from 0.22 to 0.03%, provokes only a slight decrease in A_w from 0.82 to 0.70 (see Figure 10). The dynamic evolution of A_w as a function of moisture content is linear. It is the sign of simultaneous water release and sucrose crystallization in the film. A sugar cube leaving the conditioning and stored at ambient temperature for 35 days has an A_w which is decreased from 0.82 to 0.46 (see Figure 11) and a moisture content from 0.15% to 0.02%. At equilibrium almost all crystallizable sugar in the film surrounding the crystals is crystallized. Thus, all liquid bridges between crystals in the cube are transformed into solid bridges constituted of syrup films surrounding each particle. When the sugar is agglomerated by moistening, three types of hooking are possible: the pendular state, the funicular state and the capillary one (see Figure 12). The fixation of agglomerates is achieved by drying which transforms liquid bridges into solid bridges. Drying of crystalline sugar or curing sugar cubes acts not only on the elimination of water but especially on the crystallization of sucrose in the film surrounding the crystals.

Drying of sugar cubes:

To accelerate the stability and reduce the curing duration, it is necessary to favour the crystallization of sucrose in the mother layer as well as to speed the release of water. When sucrose cubes are manufactured, moistening of crystalline sugar with water or with a saturated syrup leads to the same result as concerns water activity after drying, i.e. $A_w = 0.82$ and moisture content 0.2% for an average grain size of 0.6 mm. However, if the sugar cubes are obtained with crystals of different sizes between 0.80 and 0.25 mm and dried at 60°C, the water content after drying greatly varies.

It decreases from 0.55 to 0.06% after 15 minutes of drying for a grain size decreasing from 0.80 to 0.25 mm. Thus, drying of the sugar cubes made with fine grain is more rapid than that of large crystals, even though the latter are more porous.

On the other hand, if a sugar at normal temperature is dried, half of the duration is used to bring it to 60°C, the working temperature in the drier. To gain time and avoid this step of heating, the sugar is heated before moistening. Comparison of the kinetics of drying at ambient temperature to that of a sugar heated at 60°C prior to moistening shows a real gain of time (about 50%). Moreover, the heat of crystallization at 60°C is sufficient to evaporate the 2% water of moistening prior to moulding the cubes.

It was also found that preheated sugar is as efficiently dehydrated at high temperature (70°C) as at ambient temperature with a stream of relatively dry air (23°C, 53% R.H.). Dehydration of sugar is accompanied by crystallization which exothermic effect is sufficient to get rid of moisture provided that ventilation is made with fresh and dry air.

Evolution of sugar during storage:

Crystalline sugar is never completely dry. Depending on the drying process, it may keep an amount of moisture of 0.02% to 0.2% without any modification of Aw. It will adsorb the more water as the impurities at the surface become important. It becomes sticky and develops caking at ambient temperature especially if the amount of reducing sugars in the impurities is high. Moreover, the behaviour of crystalline sugar during the storage depends on the temperature and relative humidity of air. If the sugar is warmer than air and if there is enough flow of humid air, sugar is dehydrated because there is a recrystallization of the superficial film of syrup which releases enough heat to evaporate water. Oppositely, if the air is warmer than the sugar, dehydration is more difficult (12). A condensation of water may be observed at the contact of sugar crystals if the dew point of air is reached. This leads to the dissolution of sugar which is always more rapid than crystallization and provokes the phenomena of caking or package stickiness. One may think that the moistness and stickiness of the package for the packed sugar cubes comes from the hygroscopicity of paperboard. Actually the stickiness is due to the setting of syrup with fixation of crystals to the paperboard rather than a moistening with water. A stain of stickiness is observed on the package if temperature is above 40°C and R.H. above 85%. This phenomenon is the more rapid, the higher the temperature, which leads to think that it originates from a diffusion of sugar in the film of syrup surrounding the crystals. Besides, if the evolution of moisture content and water activity of the sugar and paperboard during the curing of sugar cubes is controlled, it is found that both the A_ws of the two products tend towards the same value, 0.70. This evolution is without any risk, as temperature did not reach the dew point. The stability and care for avoiding condensation may impose a duration of curing of 4 to 5 days. However, a good ventilation with ambient air should be sufficient. Indeed the permeability of paperboard does not constitute a restraint to the release of water vapour. Its value is very high about 700g/m₂/24h. The dehydration kinetics of a sugar cube submitted to a flow of air at 20°C is compared to a drying at 40°C and 75% R.H. While air at 20°C has a dew point at 15°C and the risk of its condensation is limited, this is not the case for air at 40°C. Thus a flow of ambient air for

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drying coupled to a treatment which allows a maximum of recrystallization of the film of syrup surrounding the crystals, should be sufficient to shorten the curing duration.

CONCLUSION

Treating a practical problem like caking or stickiness of package needs a minimum of theory. This theory is rather that of molecular interactions between water and sugar in the supersaturated solution surrounding a crystal, than that of drying. It is necessary to bear in mind the notions of thermodynamic equilibrium of adsorption of water by sugar and the influence of impurities on this adsorption. One also should keep in mind the activities or real concentrations of water and the sugar. Finally, it is to be reminded that the water-sugar interactions concern three levels : humidity of the atmosphere, the syrup surrounding the crystal and the sucrose crystal. These interactions are hydrogen bonds which are established and ruptured under the influence of temperature, heat of crystallization, concentration, etc. The transfer of water and sucrose depends on a number of obstacles like diffusion, viscosity, water de-association and the incorporation of sucrose molecules into the crystal. All these aspects of crystallization in a thin layer must be taken together with sucrose hydration, especially if the conformation of the molecular changes cannot be treated by the sole unit operation of drying. They need to be approached at a molecular level with the utilization of molecular spectroscopy techniques.

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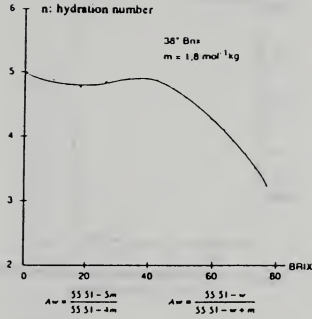
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Table 1. Effect of temperature on the water activity of a solution of sucrose at 70° Brix.

°C	Aw
0	0.94
40	0.91
60	0.83
70	0.83
90	0.83



n: Hydration number m: molality w: nm

Figure 1. Hydration number (n) of sucrose as a function of solution concentration in °Brix.

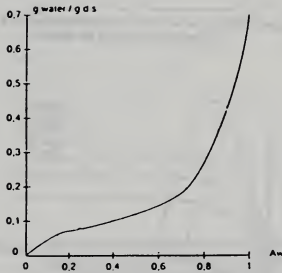


Figure 2. Water vapour sorption isotherm (25°C) of aqueous sucrose solution

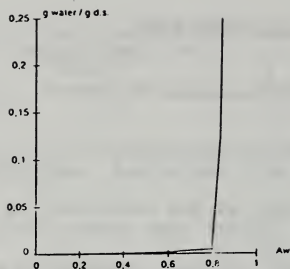


Figure 3 : WATER VAPOUR SORPTION ISOTHERM (25°C) OF CRYSTALLINE SUCROSE

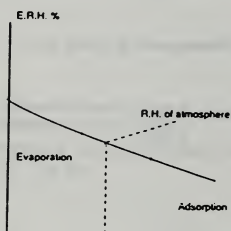


Figure 4 : EFFECT OF IMPURITY IN THE FILM SURROUNDING A SUCROSE CRYSTAL ON THE E.R.H. OF SUCROSE

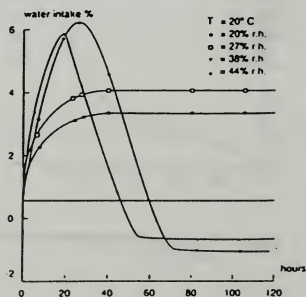


Figure 5 : KINETICS OF WATER SORPTION BY AMORPHOUS (FREEZE-DRIED) SUCROSE SHOWING RECRYSTALLIZATION AND DESORPTION AT 38% AND 44% R.H.

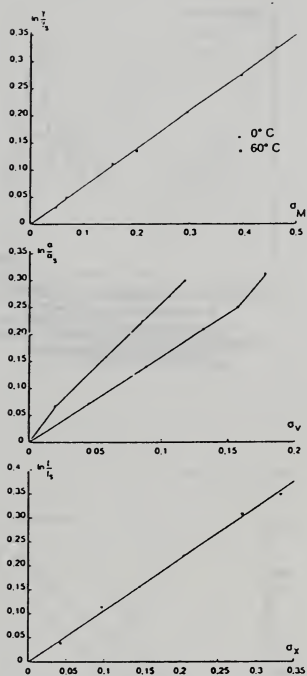


Figure 6: ACTIVITY COEFFICIENTS OF SUCROSE AS A FUNCTION OF SUPERSATURATION MOLAL SCALE (σ_M), MOLAL FRACTION (σ_x) AND VOLUME CONCENTRATION SCALE (σ_v), SATURATED SOLUTION BEING THE STANDARD STATE

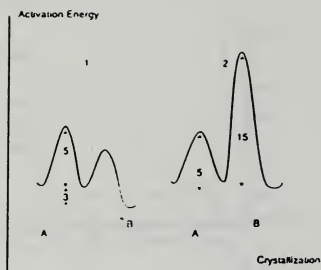


Figure 7: SCHEMATIC REPRESENTATION OF THE ACTIVATION ENERGY OF A 2-STEPS CRYSTALLIZATION PROCESS :
 1 : AT HIGH TEMPERATURE ; 2 : AT LOW TEMPERATURE
 A : DIFFUSION IN SOLUTION ; B : DEHYDRATION-DISSOCIATION AND INCORPORATION INTO THE CRYSTAL

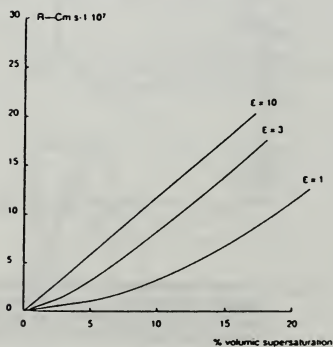


Figure 8 : EFFECT OF SUPERSATURATION ON THE RATE (R) OF GROWTH OF CRYSTALS WITH DIFFERENT DEGREES OF DISLOCATIONS

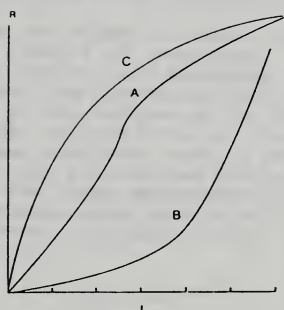


Figure 9 : CRYSTALLIZATION RATES :
C : CRYSTALLIZER ; B : SINGLE CRYSTAL ;
A : LIMITED AREA AS IN A SUGAR CUBE

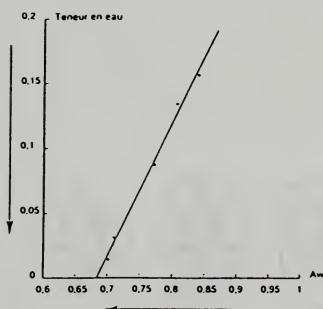


Figure 10 :
EVOLUTION OF A_w AND WATER CONTENT OF A SUGAR CUBE
(IN THE DIRECTIONS OF ARROWS DURING THE FIRST STEPS OF CURING)

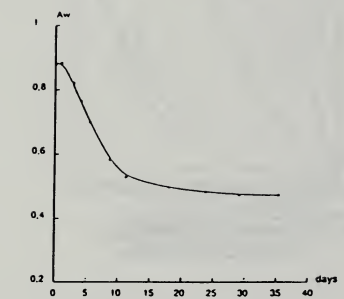


Figure 11 : EVOLUTION OF A_w OF A SUGAR CUBE
(UNTIL STABILITY AFTER 35 DAYS)



Figure 12 : TYPES OF HOOKING OF SUGAR PARTICLES IN
AGGLOMERATED MOISTENED SUGAR
(a : PENICILLAR ; b : FUNICULAR ; c : CAPILLARY)

DISCUSSION

Question: Could you elaborate a little further on the stability of amorphous sugar, during and after production?

Mathlouthi: To master the amorphous sugar: first-we must remember the lesson of last lecture of yesterday, it's much better to prevent than to cure. Not to have amorphous sugar and not to have dust are goals in conducting crystallization the right way. Not to have amorphous sugar at the surface of the dried sugar, by giving time to the sugar to recrystallize at the crystal surface is important: even if some amorphous sugar is produced at the surface during drying, 3 or 4 days are required for curing, to establish an equilibrium between the film surrounding the crystal and the crystal itself. Amorphous sugar should be dissolved in the syrup film and recrystallized, releasing water, to create a stable situation.

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NEAR INFRARED SPECTROSCOPY IN THE SUGARBEET INDUSTRY

Jan Maarten de Bruijn

CSM Suiker bv, Centraal Laboratorium, Breda, The Netherlands

ABSTRACT

During the 1992 sugarbeet campaign NIR spectra were collected using a scanning NIRSystems Model 5000 spectrophotometer in reflectance mode. Calibration curves were obtained for pol and dry substance content in brei, cossettes, wet pulp, pressed pulp respectively, and in carbonatation sludge. The correlation between the actual lab data and the NIR predicted data appeared to be remarkably good.

In 1993 the NIR instrument was extended by a transmission detector in order to develop a NIR analysis method for pol, Brix and purity in juices and syrups. A single calibration curve for each analyte was constructed, applicable for all types of liquid samples, so the whole range from raw juice up to molasses was covered at once.

Our experiences concerning NIR method development and validation will be discussed as well as future plans for implementation and application of NIR analysis in sugar factories.

INTRODUCTION

Near InfraRed (NIR) Spectroscopy enables multicomponent analysis in a variety of products, e.g. liquids, viscous samples and solids, and may be utilized for process control as well as quality control. The possibility for application of NIR analysis for these purposes in sugar manufacture has prompted several studies in both beet sugar and cane sugar industries.

Two recently organized conferences for sugar technologists and chemists, i.e. the General Assembly of ICUMSA in Cuba, Subject 8 (1), and the meeting of the CITS Subcommittee Measurement and Process Control at Tienen (3), reviewed the state of the art for application of NIR in the sugar industry. The outcome of most research programs is promising and encouraging as a result of the progress NIR hardware and software have made in recent years. However, it turns out that still a lot of method development has to be done in order to allow NIR to be implemented as a mature analysis method in sugar manufacture. Particularly the analysis errors in developed NIR methods are often much larger than would be tolerated in lab analysis. Therefore up to now NIR can not be used

for quality control of products, commercial transactions and beet or cane payment. On the other hand NIR has already proven to be a convenient method for routine and process control, as for these purposes achieving fast analysis results prevails over precision of the measurements.

In order to determine the potential field of NIR application in CSM Suiker, two years ago we bought a NIR instrument and started an explorative study. The main aims were:

- Replacement of current time-consuming and cumbersome methods, particularly those for determination of dry substance and polarization in solid samples like beet tissue, pulp and carbonatation sludge. This would allow more adequate process control with respect to sugar loss and product quality.
- Removal of lead acetate clarification in sample preparation in view of the negative environmental aspect.
- Reduction of labour requirements and/or more frequent information about the process with the same staffing.
- Last but not least, acquisition of information on the benefits and draw-backs of the NIR technique, e.g. requirements for method development, ease of operation, robustness of the instrument, speed of NIR analysis.

The results of this explorative study will be discussed in this paper. Based on (statistical) evaluation of the data, future prospects will be given for implementation of NIR in the package of analysis methods at CSM Suiker.

METHODS AND MATERIALS

NIR analysis

A bench top NIRSystems Model 5000 spectrophotometer (NIRSystems, Inc. represented by Perstorp Analytical B.V.) was used, with Sample Transport Module. This NIR instrument, scanning in the 1100-2500 nm region, was equipped with both a reflectance and a transmission detector.

NIR spectra of heterogeneous solid samples were obtained in reflectance mode using either standard sample cups for ground samples (beet brei, cossettes, carbonatation sludge) or a natural product cell for coarse samples (wet and pressed beet pulp). With the large coarse sample cell, about 60 cm² surface area of sample

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can be scanned using the sample transport mechanism. Beet brei samples were produced by the sawing machine in our tarehouses and directly packed in the sample cup, after which the NIR spectrum was obtained. Cossettes were macerated in a household blender, type Tefal Quick Foodmaster 8500. Also the particle size of wet and pressed beet pulp was reduced using the Tefal Quick Foodmaster 8500. Carbonatation sludge samples were homogenized in a household paste mixing device, type Philips Batteur HR 1480.

A 1 mm quartz cuvette was used for transmission measurement of liquid samples, i.e. juices, syrups, massecuites and molasses, after diluting the samples to a Brix in the range 10-20 g/100 g.

In order to prevent temperature affecting the NIR spectra, a temperature controller was used to adjust the temperature of liquid samples to 32 °C. Pulp and carbonatation sludge samples were cooled to ambient temperature prior to NIR analysis.

The NIRS 3 software package of Infracore International (ISI, version 3.0) was used for routine operation, calibration and prediction analysis. A 386 lap top computer Compaq LTE Lite/25C was used to collect NIR spectra at different locations; a IBM PS/2 model 90 XP486 computer was used for calibration purposes.

The NIR data were derived from single spectra, obtained by scanning the samples during 30 sec for liquids, 64 sec for beet brei and carbonatation sludge, respectively 100 sec each for pulp and cossettes.

Laboratory analysis

The lab data for polarization and α -amino nitrogen were determined by the Venema automatic analysis system in our tarehouses. The dry substance in beet brei and cossettes was determined by vacuum oven drying at 80 °C; that of wet and pressed beet pulp and carbonatation sludge was obtained after drying at 105 °C. Polarization of pulp and carbonatation sludge was carried out in lead acetate extracts of these samples. Reference lab analyses of densimetric Brix, pol and purity in liquid samples were obtained from the automatic analyzers at our factory laboratories as well as by manual analysis. All reference laboratory analyses were based on individual measurements.

Method development

Apart from the possibility to obtain validated calibration curves developed by others, NIR always necessitates the design and execution of an own method development program which should include several subsequent steps as depicted in Figure 1. Every link in the chain should be as good as possible in order to get the best

possible results out of the NIR instrument. In the next part of this paper the role of some of the crucial links will be dealt with in more detail. The theoretical background of selection of an appropriate set of samples for calibration, the mathematical treatment of the data and multivariate regression techniques involved in calibration and validation lies beyond the scope of this paper. For more specific information one is referred to the numerous textbooks and papers published on these subjects.

Statistical evaluation of data

Measurements of uncertainty are usually referred to in NIR as error. The predictive error of a NIR calibration curve or equation is characterized by the so-called Standard Error of Prediction SEP. Formula to compute SEP:

$$SEP = (\sum d^2/n)^{1/2}$$

in which d represents the difference between the predicted NIR value and the actual laboratory analysis; n is the sample size. In fact there are many different abbreviations and terms to represent the same statistic, e.g. Standard Error of Validation (SEV), Standard Error of Performance (SEP), Root Mean Squared Deviation (RMSD).

The SEP value used in the NIR field contains contributions from the NIR measurements, the reference laboratory measurements and the differences between methods. Examples of the latter type of contribution are for instance the total Kjeldahl nitrogen (KjN) determination as lab method and the various NIR vibrations of nitrogen compounds in the NIR region, and polarization measurement versus sucrose vibrations in NIR. In both cases there is not necessarily a 1:1 correlation between lab and NIR analysis.

To NIR users it is of more interest to consider the SEP value as comprising two contributions only, viz.:

- One contribution from the NIR measurement; including sample preparation and packing, etc.
- One contribution from "others", particularly those from the reference lab analysis.

Symbolically:

$$SEP^2 = s_{NIR}^2 + s_{others}^2$$

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It is essential to keep an eye on this splitting in the effort made in improving the NIR method.

RESULTS AND DISCUSSION

Beet brei

For beet brei NIR equations were developed for dry substance (ds), polarization (pol) and α -amino nitrogen (α N), based on 147 samples in the calibration set. Validation of these equations with independent sample sets (n=36 for pol, 73 for N) resulted in the following SEP values:

SEP beet brei:

ds (g/100 g)	0.19-0.26	(\bar{x} =22)
pol (g/100 g)	0.10-0.13	(\bar{x} =16)
α N (mmol/kg)	1.7-2.8	(\bar{x} =20)

As an example, Figure 2 graphically displays the lab versus NIR data for 73 beet brei samples in a validation set. At first sight the NIR errors for beet brei seem reasonably good. They appear to be excellent, knowing the repeatability and reproducibility of the reference lab analysis.

Standard deviation lab analysis beet brei:

	<u>N1 (4)</u>	<u>ICUMSA (2)</u>
Repeatability, s_r		
pol (g/100 g)	0.06	0.05-0.11
α N (mmol/kg)	0.4	
Reproducibility, s_R		
pol (g/100 g)	0.06-0.10	0.08-0.18
α N (mmol/kg)	0.4-2.0	

As the reference lab data were obtained from two different tarehouses, the moderate reproducibility of the lab analysis explains approximately 50% of the final NIR prediction error. It is obvious that NIR itself causes an additional error as well. However, without any doubt, improvement of both methods is possible; for instance by replicate analysis SEP values similar to the lab repeatability standard deviations should be attainable.

Cossettes

Only 55 samples were used to develop the calibration curves for total solids (ds) and pol in ground cossettes. Furthermore the household blender applied for macerating the cossettes appeared to be not very effective, as after blending the particle size amounted to 1-5 mm, which was far greater than in homogeneous brei. Consequently, the SEP values for cossettes (n=17 and 29 respectively) were much higher than those achieved for beet brei.

SEP grounded cossettes:

ds	(g/100 g)	0.29-0.35	(\bar{x} =22)
pol	(g/100 g)	0.27-0.30	(\bar{x} =16)

This phenomenon stresses the need for a special device to macerate the beet tissue to more homogeneous material. In other words, sample preparation of cossettes largely determines the SEP's through its substantial contribution to s_{NIR} . If homogeneous brei could be produced from cossettes, NIR prediction may be allowed simply by using the beet brei calibration curves.

Wet and pressed beet pulp

The NIR equation for ds and pol in wet pulp were derived from a calibration set containing 64 samples. The resulting SEP values from an independent sample set (n=28) may considered to be moderate.

SEP wet pulp:

ds	(g/100 g)	0.22	(\bar{x} =10.5)
pol	(g/100 g)	0.15	(\bar{x} =2.3)

The calibration set for pressed pulp contained 65 samples. The SEP values for pressed pulp, obtained with independent validation sets (n=27 and 43 respectively), were more or less comparable to those of wet pulp taking into account that the ds of pressed pulp is twice that of wet pulp and the pol of both products is approximately the same.

SEP pressed pulp:

ds	(g/100 g)	0.38	(\bar{x} =22)
pol	(g/100 g)	0.13	(\bar{x} =1.9)

Upon investigating the repeatability of the lab analysis it turned out that the lab s , was of minor importance.

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Repeatability lab analysis pressed pulp, s_r :

ds (g/100 g)	0.13
pol (g/100 g)	0.07

In conclusion, the standard error due to NIR, i.e. s_{NIR} , determined for the most part the SEP values. The origin for the relatively high s_{NIR} appeared to be related to the poor repeatability of the NIR analysis. Although repeated scanning of an individual sample (remaining in the sample cell) revealed a standard deviation in the NIR analysis comparable to the lab s_r , refilling and thus repacking of the same sample in the sample cell combined with repeated scanning, caused a substantial error.

Repeatability of NIR analysis pressed pulp upon refilling of the sample cell:

ds (g/100 g)	0.41
pol (g/100 g)	0.10

It can be concluded that standardization of sample preparation, essentially packing of the sample cell, is required in order to reduce the SEP's for wet and pressed pulp to an acceptable level. Additionally a better grinding of pulp samples, resulting in smaller and more uniform particle sizes, will further improve the overall SEP's of the NIR analysis.

Carbonatation sludge

For calibration of ds and pol in carbonatation sludge a total of 66 samples was used. Validation with 3 sample sets ($n=10$, 6, respectively 10) showed acceptable SEP values.

SEP carbonatation sludge:

ds (g/100 g)	0.5-1.1	($\bar{x}=49$)
pol (g/100 g)	0.05-0.09	($\bar{x}=0.5$)

As the so-called HCl-insoluble ash content varied between the 3 validation sets this may explain the relatively wide range of SEP's. Also the somewhat biased results for a sample set low in HCl-insoluble ash underlines this assumption; bias -0.53 for ds and +0.10 for pol. Including carbonatation sludge samples with a more equally spread variation in their composition will presumably result in more robust NIR equations.

Liquid samples

For NIR analysis of liquid samples a total of 116 samples was used for calibration. This calibration set comprised (unfiltered) raw juices, thin and thick juices, standard liquor, massecuites from different strikes, intermediate and affination syrups, and molasses. If necessary the liquid samples were diluted to a Brix of about 15 %. In this way robust calibration curves for Brix and pol were obtained in liquids from sugar manufacture, covering the whole range from raw juice up to molasses.

The NIR purity of the liquid samples was calculated from the predicted Brix and pol values. The reference lab data were obtained from different sources, i.e. the automatic analyzers at the factory laboratories, and manual analysis.

The developed NIR method was validated by 3 independent sample sets containing respectively 66, 72 and 20 samples. Figure 3 shows examples of the correlation between lab analysis and NIR calculated data. The achieved SEP's for NIR analysis of liquid samples appeared to be promising.

SEP liquids:

Brix (g/100 g)	0.09-0.12	(\bar{x} =16.2)
pol (g/100 g)	0.07-0.14	(\bar{x} =14.0)
purity	0.7-1.0	(\bar{x} =86.4)

Though the SEP's may considered to be remarkably good for such a robust calibration, we were not completely satisfied as an SEP value of almost 1 unit for the prediction of purity is not precise enough for process control purposes. Therefore we again carried out a statistical study on the origin of the error in NIR prediction. First of all we determined the repeatability of the lab measurements for Brix and pol. It appeared that the very good (low) s_e values of the lab analyses could not explain the observed error in NIR analysis. On the contrary, a collaborative study in which weekly composite samples of both thick juices and molasses were analyzed at different CSM laboratories revealed that reproducibility was not as good as expected. The standard deviations of the reference lab analysis are recorded below.

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Standard deviation lab analysis thick juice and molasses:

Repeatability, s_r

Brix	(g/100 g)	0.013
pol	(g/100 g)	0.015
purity		0.11

Reproducibility, s_R

Brix	(g/100 g)	0.03-0.07
pol	(g/100 g)	0.03-0.11
purity		0.35-0.61

It must be noted that the above-mentioned standard deviations of the lab analysis have been calculated at the same Brix and pol level as in the NIR validation sets.

As the reference lab data used for NIR calibration were derived from different instruments we have to deal with reproducibility here. The observed moderate lab reproducibility explains part but not all of the SEP in NIR prediction. Apparently, as a consequence of the robustness of the calibration, an additional NIR error was introduced as well. Nevertheless it will be possible to improve the precision of the NIR analysis easily by using lab analysis results from only one instrument for calibration purposes. This approach will not include the between-lab variation in the development of NIR equations. Independently, the between-lab variation should be the subject of further study.

SUMMARY AND CONCLUSIONS

Concerning the reflectance of heterogeneous solid samples, in most cases sample preparation is the main source of error in NIR prediction. A better standardized procedure certainly will improve the analysis results, after which NIR will be able to compete with existing methods for dry substance and pol determination. In particular, an efficient blender or mixing device, producing homogeneous samples, and standardization of packing the sample cell will bring NIR a step forward to application in the sugar industry.

By NIR transmission of liquid samples, global calibrations for Brix and pol were obtained, covering the range from raw juice up to molasses. The error in NIR prediction of Brix, pol and calculated purity appeared to depend largely on the (moderate) reproducibility of the reference lab analysis. For the 1994 sugarbeet campaign it is planned to develop new calibrations for liquids using only one lab instrument for Brix and one lab instrument for pol analysis in

order to rely on more precise reference data. Based on the encouraging results, we are aiming at application of NIR transmission in our factory laboratories for routine control of liquid samples. Then, apart from the above mentioned improvement in calibration, both automatic scanning of samples and retrieval/recording of the predicted data are required. A feasibility study will be undertaken.

From the underlying explorative study it can be concluded that the NIR prediction of dry substance content and polarization in a wide variety of liquid and solid samples look very promising. In our opinion, application of NIR at CSM Suiker seems to be within reach. It may be considered a challenge to further improve the precision of the NIR methods to meet the required standards.

Method development appeared to be the bottle-neck in establishing NIR analysis as a mature measurement technique. Specific knowledge on analytical chemistry and chemometrics is needed as well as persistence and faith. Once all obstacles in method development are removed, NIR offers a fast and versatile analysis method which can easily be operated without any specific knowledge on NIR at all. Maintenance of NIR instrumentation and the necessity for a frequent calibration-update or recalibration may be encountered as additional problems; however, we do not have experience in this area up to now.

ACKNOWLEDGEMENTS

The author wishes to thank Chris van Emmerik for his contribution to the development of NIR reflectance methods and for many stimulating discussions. Rob Ruys of our laboratory is acknowledged for recording the NIR transmission spectra.

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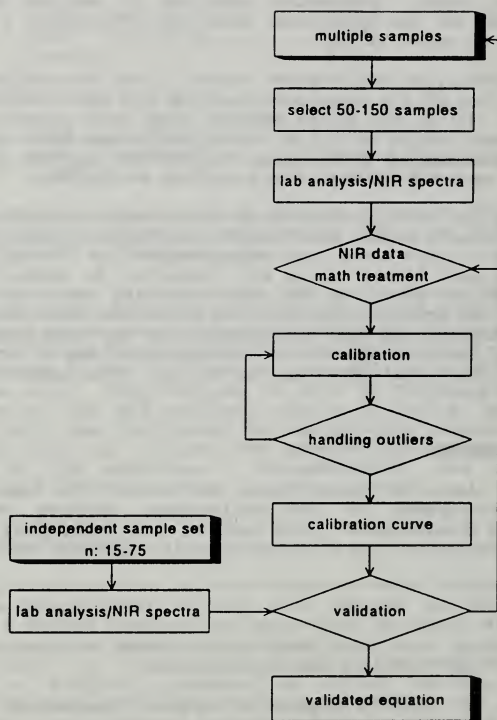


Figure 1. Principle of NIR method development at CSM Suiker.

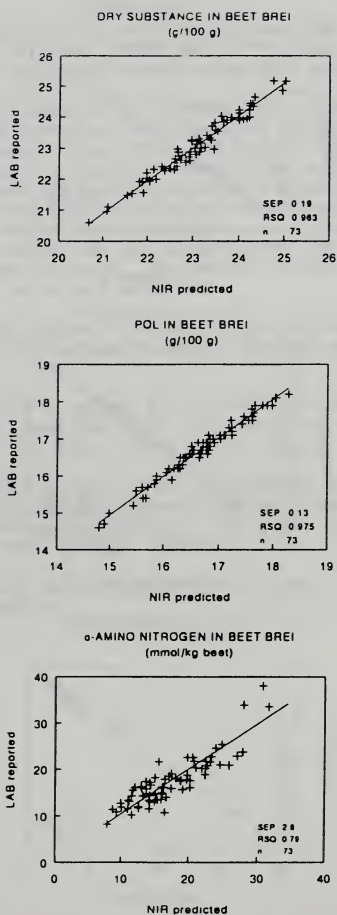


Figure 2. Validation of NIR calibrations for dry substance, pol and α -amino nitrogen in beet brei.

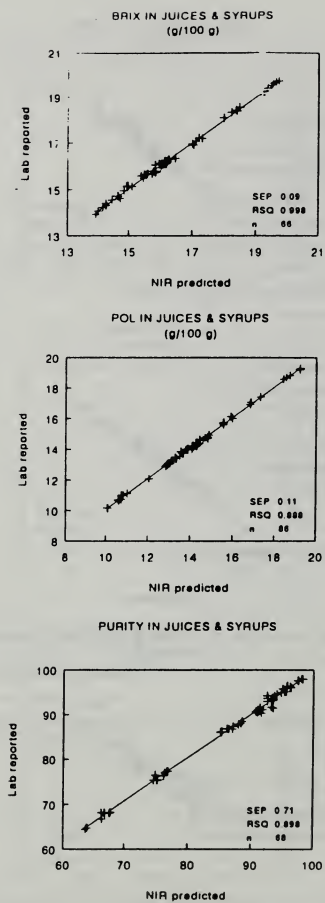


Figure 3. Validation of the global NIR calibration for brix and pol in (diluted) 'juices and syrups'. The NIR-purity is calculated from the NIR predicted brix and pol values.

DISCUSSION

Question: Thank you for this very well-presented paper. With regard to the automated analysis scheme shown on your last slide, would you comment on the expected benefits of NIR analysis at CSM?

de Bruijn: There are several advantages: one important advantage is the removal of lead acetate clarification which is quite costly in our country (the cost of chemicals and paper, and the disposal cost of this material costs about Dfl 20,000 for one factory lab during one campaign). There is less handling in sampling preparation for NIR, so time is gained and labor requirement reduced in the factory laboratory. There are other advantages - the possibility of adding additional calibrations in the same time, for invert sugar, for example, and for ash content, in special products. You can expect to replace further standard analyses.

Question: Payment to growers is always a difficult problem. Do you think your growers will be more easily satisfied with NIR measurements?

de Bruijn: No, I don't think so, in our country. I still regard that as a question, but for some countries it may not be possible in the foreseeable future.

Question: A comment: your normal laboratory errors, as shown in your reference analyses in slides, are much smaller than those in many factories in other countries. Are those your normal standard errors, compiled over several years?

de Bruijn: These are the normal errors in our standard lab methods from the last campaign.

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ON-LINE NIR FOR SUGAR-HOUSE SYRUPS: A TECHNOLOGICAL STEP TOO FAR?

Russell L. Jones, Geoff Parkin and Charles W. C. Harvey

Scientific and Technical Services Laboratories, British Sugar
Technical Centre, British Sugar plc, Colney, Norwich, United
Kingdom

ABSTRACT

British Sugar considered that the greatest potential benefits from NIR would come from on-line continuous measurement of sugar and solids in sugar house syrups. Preliminary experiments showed that laboratory NIR could perform these analyses, so a true on-line system was tested.

Earlier work with thick juice and high green syrups was encouraging, but cost/benefit analysis showed that the instrument was far too expensive to justify for use on only one syrup. To increase the versatility, a manifold was constructed to enable standard liquor, high green and molasses to be analysed by the instrument in turn.

Calibration for solids proved acceptable. Attempts to produce a calibration for apparent purity, either directly, or by calibrating for polarisation and calculating the apparent purity from the solids and sucrose, were unsuccessful. This was due to three main problems:-

- a) The apparent purity variation of the samples was very low and was of a similar order to the precision of the reference technique.
- b) The sucrose concentration, partly as a consequence of (a), was closely correlated with the solids concentration, making the derivation of a sucrose calibration very difficult
- c) The region of the spectrum which was used shows relatively little detail

It was concluded that the NIR technology available at the time of this work was not adequate to permit true on-line measurement of sugar syrups for solids and sugar.

INTRODUCTION

In any continuous production process, monitoring and control are of paramount importance. In the beet sugar process efficiency must be maintained by intelligent use of accurate and timely analysis to indicate changes in the process. Recent developments in laboratory instrumentation has provided improvements in both speed and accuracy of analytical tests and also has enabled sugar factories to operate with fewer personnel dedicated to analysis. There is, however, still considerable scope for further improvement and NIR analysis has the potential of producing radical change in this area, as it has the capability of providing rapid results for several analyses simultaneously with little or no sample preparation.

In the British Sugar context, however, the benefit of replacing standard laboratory instrumentation with a laboratory NIR would be small. The NIR would provide faster analysis, but would still require collection and some preparation of samples. More importantly, the standard laboratory instruments would still be required for calibration and checking purposes. British Sugar considered that the most cost effective goal of NIR was true on-line measurement as this would enable control of the process in real time; hence it was decided not to pipe samples to the laboratory and use a laboratory instrument (3), but instead to measure sugar house syrups continuously with no sample collection or preparation.

OBJECTIVE

To develop an NIR system for on-line measurement of Brix and apparent purity of standard liquor, high green syrup and molasses to provide data suitable for process control in real time.

INSTALLATION

The instrument used throughout was the NIR Systems model on-line 5500 using a fibre optic bundle and transreflectance probe (Figure 1).

Previous work (1) had shown that this NIR instrument was capable of producing satisfactory results for Brix and apparent purity of high green syrup with an on-line installation at Wisington factory, although the system was not put to practical use after the development stage.

At the time, it was not possible to multiplex the NIRS 5500 fibre bundles so, in order to monitor the three sugar house syrups mentioned above, a manifold was designed and constructed (Figure

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2). This manifold was installed at York factory during the 1992-93 campaign and the design allowed all three juices to flow continuously and be presented to the instrument in turn. Provision was also given for steam to be applied to the pipework for cleaning the system. The valve positioners were connected to the factory's Honeywell control system, thus ensuring that illegal combinations of valves did not occur and permitting automatic switching of syrups for scanning.

Earlier work had indicated that different calibration equations were necessary to predict results for different syrups, so a means to select the appropriate equations when different syrups passed through the instrument was needed. Two possibilities were considered:-

- a) Connect valve switching logic to NIR computer
- b) Use the NIR spectra themselves to identify the syrup being scanned and automatically apply appropriate calibrations

The availability of IQ² Software, the NIRSystems program for identification, qualification and quantification of samples led to the decision to use option (b), as it allowed a much simpler installation to be engineered. This software compares collected spectra with a library of reference spectra and can provide statistics showing how closely the new data matches the library spectra. Thus these identification and qualification stages may give further useful information about the spectrum, beyond the results generated from the calibration equations.

The intended operating logic was as follows:-

- a) All three syrups flow continuously through bypass loops.
- b) Valves open to allow juice to flow past the sample head.
- c) Instrument scans continuously to produce one spectrum from every 256 scans, i.e. one spectrum approximately every 4 minutes.
- d) The spectrum compared to library with IQ² software.
- e) If the spectrum is identified, then the appropriate calibration equations for that syrup are selected.
- f) Scanning continues and the sequence is repeated.
- g) After a standard time interval, the valves close for one syrup and the valves open for the next duty.

Obviously some spectra contain information from two sample types, as one set of valves opens and another closes, but in practice these transitional spectra were correctly identified by the software as unknowns, and thus rejected. It was apparent that the second and subsequent spectra produced after valve switching did not contain any elements of the previous sample. The flow rates

were sufficiently fast to ensure traces of previous samples were flushed consistently. The synchronisation of valves and NIR scanning was set such that only one spectrum was lost when syrups changed.

RESULTS AND DISCUSSION

The data was collected over two periods, during the Campaign from January to March 1993 and during the Juice Refining Operations (Juice Run) in April and May 1993. The calibrations were generated using the samples collected during the Campaign. The procedure for laboratory measurements and production of calibrations is given in Appendix 1. The samples collected during the Juice Run period were used to check these calibrations.

Problems with flow of high green syrup to the manifold resulted in few usable samples or spectra for this syrup, so only molasses and standard liquor will be discussed in this report.

The initial approach to calibration for Brix and purity of syrups was to apply equations generated in the previous work (1). These proved to be inappropriate for both analytes, possibly because of the hardware and software changes and the installation differences, so the whole procedure of collecting data and deriving calibrations was started anew.

In the following figures the Standard Error of Prediction (SEP) is quoted. This is calculated as the square root of the mean of the sum of the squares of the differences between the lab and NIR results. The difference between lab and NIR results is known as the residual.

Brix Calibration

Brix calibrations were derived which were similarly successful for both syrups. This is illustrated in Figures 3, 4, 5, which show the predictions of molasses Brix, and in Figures 6,7,8 for standard liquor Brix, both for the campaign period. The agreement with refractometric Brix was similar to that encountered in earlier studies. Note, however, that the calibration equations adopted for the two syrups were different (Table 1). The Juice Run samples confirmed that the Brix calibrations were reliable (Figure 15).

Apparent Purity Calibration

Attempts were made to treat apparent purity as an independent constituent.

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There was, however, very low variation in the purity of the syrups tested, and this produced a problem. The precision of the apparent purity method was approximately 0.3% for careful duplicate analysis by an experienced analyst. The whole range of purity for a given syrup was only about ten times this, approximately 3%, and this ratio of calibration method precision and analyte variation is insufficient for the production of entirely satisfactory calibrations.

However, an apparent purity calibration was initially thought to be possible for molasses. The results obtained when this calibration was used to predict the whole set of campaign molasses samples are shown in Figures 9,10,11. A few outliers were still present, however, even after accuracy of the laboratory analysis was confirmed.

Unfortunately, the reason for the apparent success with molasses was that, for the subsets chosen for the generation of calibration equations, Brix and apparent purity are by chance correlated. This gave rise to a calibration where the Brix information in the spectrum is generating the apparent purity result. The NIR prediction produces a low apparent purity result for a higher Brix sample, and the outliers are due to samples which quite legitimately do not fit this pattern (Figures 17,18). The Juice Run samples confirmed that the molasses apparent purity calibration was unsatisfactory (Figure 16).

It was not possible to produce a satisfactory apparent purity calibration directly for standard liquor and no false correlation of Brix and apparent purity was apparent. The best calibration obtainable for standard liquor produced unsatisfactory results when tested on the whole campaign data set (Figures 12,13,14).

As it was found to be impossible to produce an apparent purity result directly from the NIR spectra, attempts were made to calibrate for sucrose.

Sucrose Calibration

Since sucrose concentration for any given syrup is closely related to Brix, it proved to be difficult to derive a calibration which would produce a result for sucrose independent of the dilution (Figures 19,20,21). Despite this, this route gave some success, in that a wavelength was found that gave a better correlation for sucrose than it did for Brix. However, the results generated from this calibration were unsatisfactory for our purposes (Figures 22,23).

Syrup Identification - The IQ² Program

This program was used to identify the syrup presented to the instrument; it was found to give reliable recognition from a small library.

It was hoped that this spectral identification software would enable more than just selection of the appropriate calibration equations. The spectra collected during the campaign were divided into subsets representing, for example, "in specification" and "out of specification" for any one syrup. The stored spectra were then processed using this program to evaluate which category the spectra fell within. This attempt to produce a pass/fail test for syrups based on spectral differences was not a success. The initial library testing "validation" stage showed that there was insufficient difference between spectra for confident separation into pass/fail sets. Many attempts were made to produce library subsets which would enable this qualification program to provide some extra function in addition to identification of samples, but without success.

CONCLUSIONS

Although the diversity of spectra used to generate the libraries was quite limited, the IQ² program gave correct identification of standard liquor, high green and molasses.

The on-line measurement of Brix for standard liquor and molasses worked satisfactorily for our purposes.

The measurement of apparent purity for all syrups at York was unsuccessful.

The direct measurement of polarisation was also unsuccessful.

FUTURE WORK

Recent discussions with personnel at the Institute of Food Research has resulted in a feasibility study around the use of a bench instrument coupled with multiple fibre-optics for use on on-line process control (2). This would enable wavelengths of up to 1700 nm to be used in conjunction with comparatively inexpensive fibres and instrumentation.

Appendix 1

Sample Collection, Lab Analysis and Calibration Procedure

Sample collection

The samples for laboratory analysis were collected directly from the manifold from the valves as shown in Figure 2. They were collected into labelled bottles in batches and analysed immediately.

Laboratory Analysis

Brix

All measurements were made using an Index Instruments GPR11-37 refractometer. This instrument has a precision of 0.1 and an accuracy/reproducibility of 0.1 degrees Brix. Most samples were measured in duplicate, but since reproducibility proved to be very good, later samples were measured once. High green samples are supersaturated at room temperature therefore they were diluted before measurement.

Apparent Purity

This was measured using a combination of refractometer and polarimeter. The refractometer was similar to the GPR above, except that precision is 0.01 degrees Brix. The polarimeter used was an Optical Activity AA101 (598.44nm) with a resolution of 0.01 °Z. Basic lead acetate was used as a clarifying agent. All measurements were made in duplicate.

Sucrose

This was measured by polarimetry using an OA AA101 instrument as above.

Calibration

Some calibration work was carried out with all syrups together, but as we found in earlier work, the best approach was to deal with them individually. Sets of spectra for each syrup for the whole of the campaign period were examined for calibration. These sets were divided into subsets for calibration and verification. Correlation and standard error results shown on the attached figures relate to the whole of the original sets.

Juice run samples were used to test calibrations.

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Table 1. Calibration details.

	Brix	Molasses Purity	Sucrose	Standard Brix	Liquor Purity
Calibration maths	2nd Derivative	1st Derivative	1st Derivative	1st Derivative	Partial Least Squares
Variables	1	2	2	1	225
Wavelength(s)	960	956/968	996/812	982	4 loadings
Correlation for calibration set (r)	0.97	0.97	0.94	0.97	0.80
SEP for Cal. set	0.43	0.16	0.40	0.45	0.41

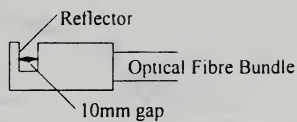
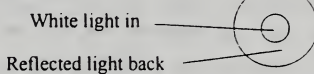
Analyser Probe**Optical Fibre**

Figure 1. Analyser probe and optical fibres.

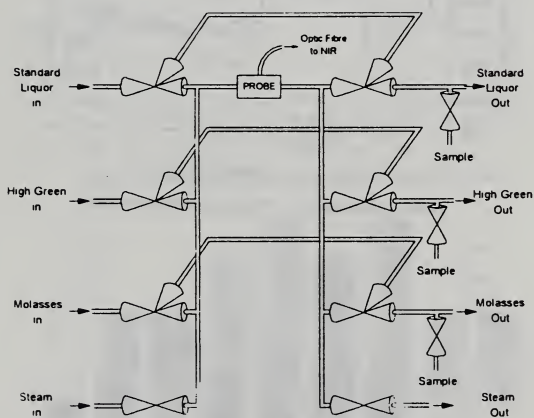


Figure 2. Manifold arrangement for NIR scanning of 3 sugar syrups

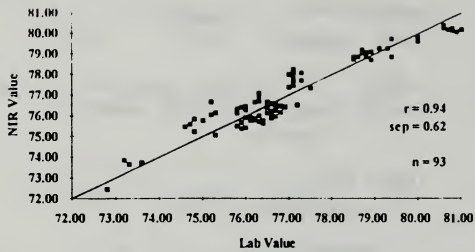


Figure 3 Molasses Brix - NIR value against Lab Value

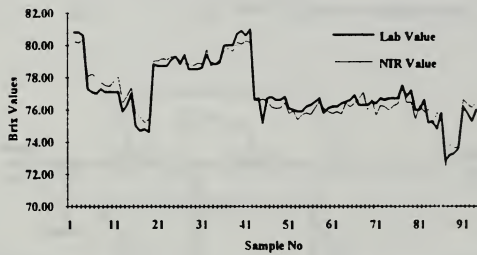


Figure 4 Molasses Brix - NIR and Lab Values over Time

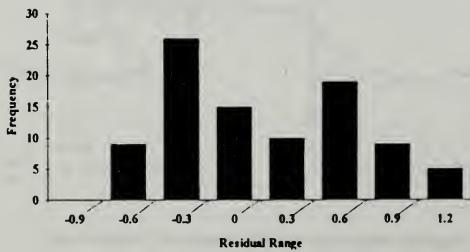


Figure 5 Molasses Brix - Frequency of Residuals

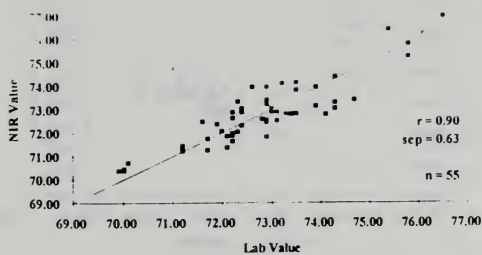


Figure 6 Standard Liquor Brix - NIR Value against Lab Value

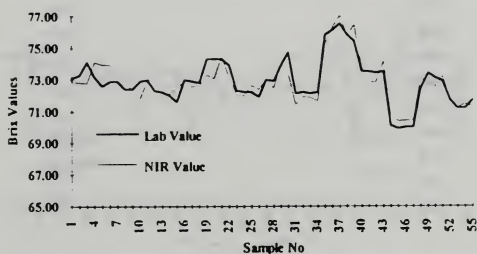


Figure 7. Standard Liquor Brix - Lab and NIR Values over Time

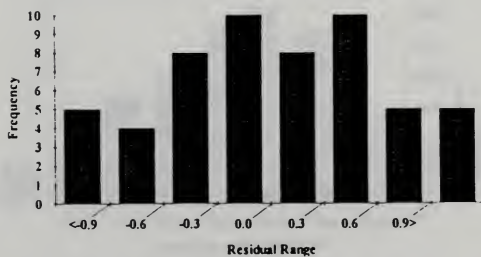


Figure 8 Standard Liquor Brix - Frequency of Residuals

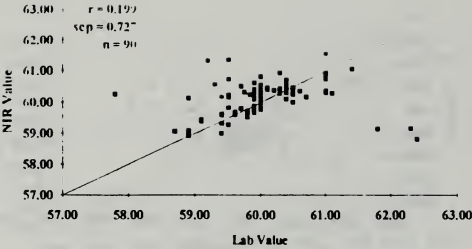


Figure 9. Molasses Purity - NIR Value against Lab Value

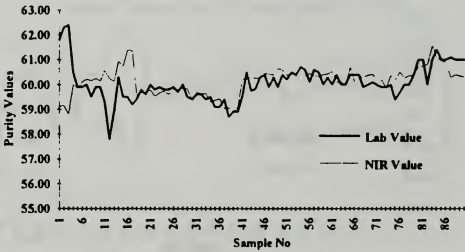


Figure 10. Molasses Purity - Lab and NIR Values against Time

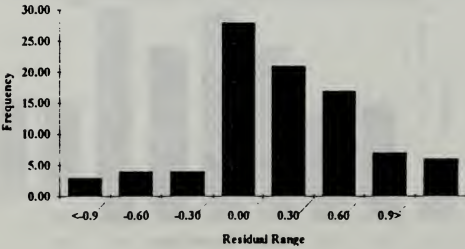


Figure 11. Molasses Purity - Frequency of Residuals

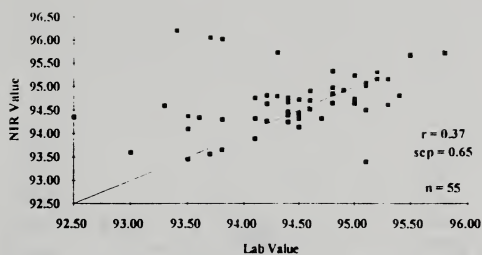


Figure 12. Standard Liquor Purity - NIR Value against Lab Value

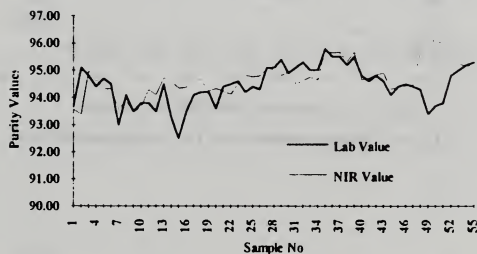


Figure 13. Standard Liquor Purity - Lab and NIR Values against Time

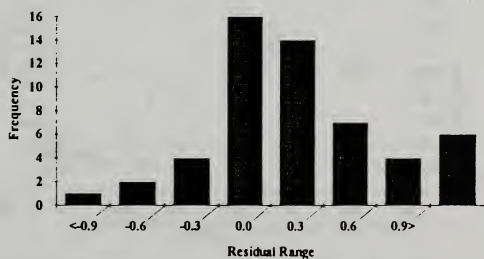


Figure 14. Standard Liquor Purity - Frequency of Residuals

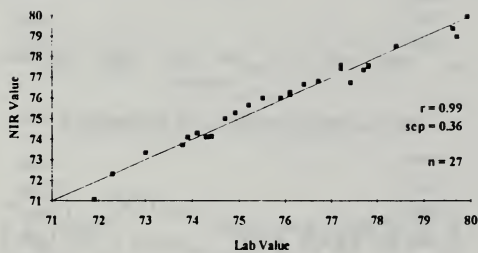


Figure 15 Juice Run Molasses Brix - Lab Values against NIR Values

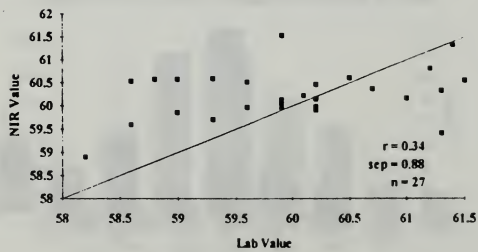


Figure 16 Juice Run Molasses Purity - Lab value against NIR value

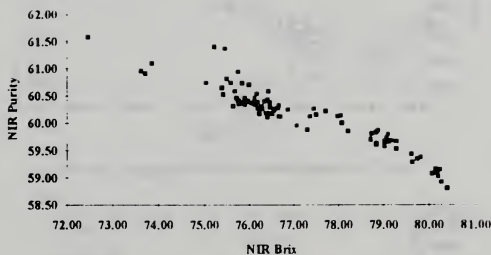


Figure 17 Molasses Brix against Purity - NIR Values

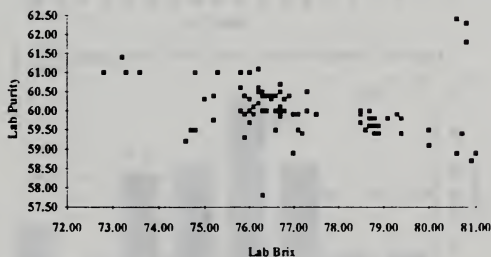


Figure 18 Molasses Brix against Purity - Lab Values

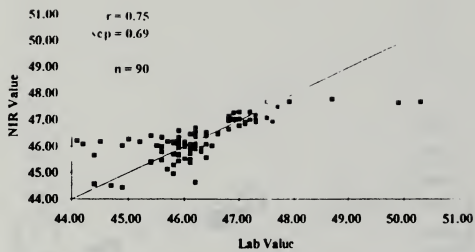


Figure19 Molasses Sucrose - NIR Value against Lab Value

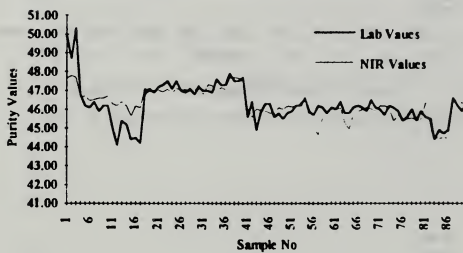


Figure 20. Molasses Sucrose - Lab and NIR Values against Time

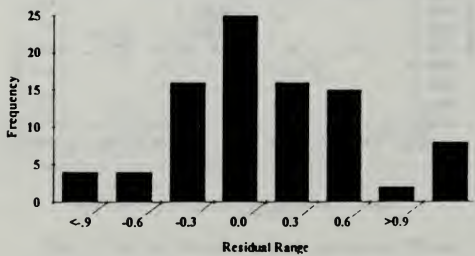


Figure 21 Molasses Sucrose - Frequency of Residuals

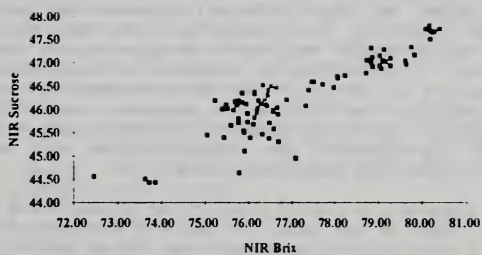


Figure 22 Molasses Brix against Sucrose - NIR Values

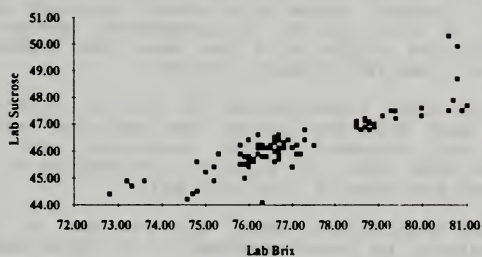


Figure 23 Molasses Brix against Sucrose - Lab Values

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DISCUSSION

Question: Would you please describe further the procedure for sampling for the spectra obtained by NIR.

Jones: In the laboratory, a sample is collected in a bottle and is ready for analysis. On line, it's all a matter of timing: we have the instrument scanning continuously, and the syrups flowing continuously, so we would collect a sample during the period of scanning so that it would not be confused with spectra collected immediately before or after. We ensured that we were getting reproducible results by taking 4 spectra, collected from 4 successive samples, and doing laboratory analyses on the 4 samples, to check that the spectra were very similar for very similar samples. That seemed to work very well.

Question: The Standard Error of Prediction classically consists of two components: one is the error in the NIR measurement, the other is from other (reference method) sources. There can be differences from the reference method in the sucrose measurement because the NIR analysis is using appropriate wavelengths to measure sucrose, while the reference method is usually polarization. Polarization is the sum of the rotation of light by all optically active substances, not only sucrose but also raffinose and also invert sugar. You may have had some problems with that in the development of calibrations. In our country (The Netherlands) and, I presume, in the United Kingdom, there is a steady increase in raffinose concentration in lower purity (afterproduct) syrups during the campaign. Raffinose starts at a low level and increases as the autumn goes on. Can you comment, please.

Jones: I agree that polarization is not an absolute method. However, we were treating syrups individually, with separate calibrations for standard liquor, molasses, etc. - we were comparing like with like. It would be interesting to use HPLC to measure sucrose and compare that with pol.

Question: What statistical model did you use to create your regression analysis in linear regression, multiple linear regression, or partial least squares (pls)?

Jones: We tried both. The Brix calibrations worked best with single or two wavelength linear regressions. For purity, we tried PLS; that was our approach. We considered that purity was not a straightforward measurement - it's not a single compound and you can't say that a single (or several) wavelength(s) will relate to this compound. So, we used PLS because it used the whole spectrum, but this was not successful. The slide we used was based on a two-wavelength regression - that was better than the PLS measurement.

Comment: One of your points for future work was wavelength range. You were restricted to wavelengths under 1100 nm; we've had better success using wavelengths above 2000 nm, and I believe the next paper will also address higher wavelengths.

Comment: In response to a request from the Chair, I can say a few words about our experience with an FT-IR instrument (mentioned by the speaker for future work). According to our results on analysis of levels of glucose and fructose, the FT-IR instrument appears to be a much more reliable instrument for our purposes. Our samples were fructose/glucose solutions. Unfortunately, the manufacturers of the instrument, from France, were not able to attend this conference. I hope that we can publish our results later, and discuss them. The spectral information, as mentioned earlier, is more clear in the mid-IR region. I think the FT-IR instruments will be very useful and powerful for solving laboratory problems and analyzing complex solutions.

Proceedings of the Conference on Sugar Processing Research,
Helsinki, Finland, August, 1994

THE APPLICATION OF NIR SPECTROPHOTOMETRY IN THE CANE SUGAR REFINERY

Phil Guglielmi and Debbie Anderson

Redpath Sugars, Ltd., Toronto, Ontario, Canada

ABSTRACT

The applications of NIR spectrophotometry for the analysis and monitoring of in-process samples and finished products in the cane sugar refinery were evaluated at Redpath Sugars. NIR can be a very valuable and versatile analytical tool in the refinery and has the potential to replace many time consuming analyses. It can also be used easily by both lab employees and operators.

In this evaluation, NIR calibrations were developed for measuring parameters such as Brix, pol, colour, ash, moisture and invert levels as required for a variety of samples, using conventional methods of analysis as reference. The prediction performance of these calibrations indicates that many conventional analyses used in the refinery can be handled by NIR with minimal effects on the quality of data used to support process control. In general, calibrations specific to a particular product stream worked better than generic or universal calibrations. Some of the issues and problems encountered during the calibration process are discussed.

The implementation of NIR analysis in a refinery process control laboratory environment is reviewed along with issues related to determining the best sample streams to monitor with NIR; lab employee training and utilization of the instrument; managing NIR generated data; and operator utilization of NIR analysis. There is a need for on-going maintenance of the calibrations by regular validation checks and the addition of new samples to expand the reference data base. Possible extensions to on-line applications are also considered.

INTRODUCTION

The cane sugar refinery relies on constant monitoring of in-process and finished product streams in order to maintain process control, manage plant efficiencies, and ensure on-going product quality. In general, to support process control requirements, the plant control laboratory utilizes a variety of standard gravimetric and wet chemical methods of analysis to evaluate samples from all key stages in the refinery operation. While these standard methods are reliable, they are time consuming and require trained laboratory

personnel and as such, cannot accommodate real-time control testing requirements.

Near Infrared Spectrophotometry (NIRS) has emerged over the past several years as an alternative analytical tool for the sugar refinery laboratory. The benefits of NIR are speed of analysis and ease of operation which allows for faster utilization of sample data for process control which can be generated by operators as well as lab personnel. Since calibration of the NIR is based on standard reference methods using real refinery samples, analytical errors using NIR methods would be comparable to the errors currently experienced with the standard methods.

Redpath Sugars acquired a NIR spectrophotometer in 1993 with plans to implement this technology in the refinery control lab and also to evaluate longer term on-line analytical applications. A detailed evaluation of potential NIR applications was conducted and the purpose of this paper is to give a general practical overview of this laboratory's approach and experience with calibration, analysis, and implementation of NIR using applications for analysis of re-melt syrups, liquid sugar products, and in-process syrups as examples.

MATERIALS AND METHODS

A model 6500 NIR system from NIR Systems was used in this evaluation. The instrument was equipped with a sample transport module suitable for handling glass cuvettes for liquids and a standard sample cup for solids. All liquid samples were analyzed neat or diluted with water as required in transmission mode while solids were analysed in reflectance mode without any sample treatment. Data collection and all subsequent mathematical treatments were performed with the NSAS software version 3.25 supplied with the instrument.

The reference methods used to analyze the various refinery samples to support the NIR calibration process are as follows:

<u>Parameter</u>	<u>Reference Method</u>
Brix	Refractometer
Pol	lead clarification
% Invert	Lane & Eynon titration
Colour	ICUMSA 420 (neutral pH)
Ash	Conductivity
% Moisture	Oven drying 105°C

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All samples used for calibration were obtained from the refinery and analyzed by NIR within 24 hours of analyzing them using the reference methods.

The sample streams evaluated represent areas where NIR analysis could provide faster information for process control; provide operators with capabilities to do their own analyses; and eliminate the use of chemicals required for analysis.

CALIBRATION

Since NIR is affected by all constituents of the sample matrix, calibration must be done with actual samples rather than primary standards so that the matrix effects can be represented in the calibration. Therefore, a large number of samples need to be analyzed during this process in order to generate good working calibrations that can be reliably used in refinery control testing.

The calibration process basically involves a preliminary NIR analysis of the sample; collecting NIR spectra for the samples; developing the calibration model for the samples using the NSAS software and second derivative spectra; validating and testing the calibration; and establishing operation files for routine use in testing. Some of the main elements of this process experienced during the Redpath evaluation are discussed as follows.

Preliminary Sample Review:

A preliminary NIR analysis of the sample is required to determine sample handling requirements, the quality of the NIR spectra, and the optimum wavelengths to use for calibration. The time for this preliminary review is well spent since many simple items - such as the correct sample cuvette size; the need for dilution etc. - which will affect the quality and application of the NIR analysis can be evaluated before a lot of time is spent on calibration analyses.

For most refinery samples, NIR will measure sucrose, invert sugars, water, and colour in some arrangement depending on the sample and measurement requirements. Figure 1 shows the NIR of spectra of 50% solutions of sucrose, fructose, and glucose in water and illustrates the general NIR wavelengths where these components have absorbance. It is important to note that these wavelengths will be affected by each sample matrix and must be confirmed by reviewing the actual spectra of the samples of interest. Water shows good absorbance in the 1410 and 1920 nm areas while sucrose, fructose and glucose show absorbances in the areas of 1650 to 1750 nm and 2150 to 2300 nm. The second derivative of this spectrum is shown in Figure 2 and shows the areas where the sugars can be resolved. Fructose has a strong absorbance at 1690 nm and this area can be

used to measure invert sugar. Other parameters such as colour can be monitored in the visible end of the spectrum - 420 to 700 nm.

An important consideration during this preliminary review is the handling and analysis requirements for the sample. This requires the determination of the best sample cuvette size to use based on the actual conditions where the NIR will be used. In this evaluation, we determined that a 4 mm cuvette was the minimum size that could be used for liquids in order to accommodate handling and cleaning requirements for viscous liquid samples.

Collecting NIR Spectra for Calibration:

The samples used for calibration must come from the refinery and the composition must reflect a wide enough range so that the calibration can accurately be applied during all operating scenarios in the refinery. It is good practice to review the error for the reference method and for the analysts who will be evaluating the samples in order to ensure that the best possible information is generated for the calibration sample set.

Since the refinery is always striving to run at steady state conditions, it is often difficult to obtain enough samples with a good range of composition. There is the opportunity to modify the composition of some samples in the lab in order to obtain the required compositions however, this was not attempted in this evaluation. Figure 3 shows the distribution of samples used for the POL calibration for remelt syrups developed in this evaluation. Whenever possible, this rectangular type of distribution should be used since it will equally represent all values in the calibration. As well, the distribution should be monitored frequently during the calibration process so that the range of compositions can be altered if needed.

The actual number of samples required for the calibration depends on the characteristics of the sample and on the constituents being measured. In this evaluation, the calibration model was developed and tested at approximately 50 sample increments in order to determine how well the calibration was working. In general, for most of the refinery applications evaluated, the good working calibrations contained between 150 and 250 samples.

Developing the Calibration Model:

This is a critical step in the calibration process and also the most complicated step for the refinery lab staff. NIR calibrations are based on the 2nd derivative of the absorbance data in order to compensate for overlapping absorbances and other matrix effects. Scatter plots of the NIR calculated values vs the reference

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concentrations (using the 2nd derivative data) are produced to develop the calibration model which is the mathematical relationship between the absorbance of the sample and the concentration of the component of interest. The correlation coefficient (r) and the Standard Error of Calibration (SEC) are the main statistics which indicate the quality of the calibration.

The NSAS software allows for the determination and elimination of any outlier samples - i.e. samples which are significantly different than the others in the set - from the calibration set in order to improve the calibration. Outliers could result from an error in the reference analysis or a matrix effect that does not occur at a high frequency and which is not well represented in the calibration set. Attention is required when manipulating these outliers since they may reflect conditions which should be in the calibration once it is working in the refinery control lab. It is recommended that the spectra of the outlier be reviewed before it is eliminated from the calibration in order to determine if the outlier is due to a matrix condition.

The calibration model is based on either a single wavelength regression analysis or multiple wavelength analysis such as linear summation or partial least squares (PLS) analysis. The details of these statistical techniques is beyond the scope of this presentation; however, a good understanding of the principles of these techniques is required in order to obtain optimum results from the calibration set. Parameters such as Brix, moisture and colour, which have unique absorbances at specific wavelengths, can be calibrated using linear regression at a single wavelength. Calibrations for POL, sucrose and invert sugars require PLS or linear summation so that overlapping absorbances (refer back to Figure 2) can be compensated for. For example, during this evaluation, PLS over the range of 1650 to 1750 nm was found to be best for measuring POL in many different samples. Using a narrow wavelength range for the PLS model was optimal since using a wider range of the NIR spectrum generally resulted in a poorer calibration as illustrated in the following example:

POL calibration for re-melt syrups:

PLS range	1650 - 1750 nm	1000 - 1800 nm
r	.989	.979
SEC	.926	1.34

Validating and Testing the Calibration:

It is important to remember that a NIR analysis is a prediction of the composition of a sample based on the calibration information. Therefore, once a calibration is calculated that yields a good correlation and SEC, it is necessary to test the calibration for

good prediction capabilities. This is accomplished by analyzing a separate set of samples (which are not in the calibration set) using NIR and the standard reference method and comparing results. The important prediction statistics are the correlation coefficient (r) and the standard error of prediction (SEP). In theory, the SEP should always be very similar to the SEC for a valid calibration. However in practice, the SEP was always found to be higher than the SEC for a good working calibration.

In some cases, a "good" calibration did not necessarily mean that it would predict well. The reasons for this are most likely a poor sample range in the calibration set, the wrong calibration model, or some physical change in the sample. For example, a specific calibration developed for affination syrup showed $r = .990$ and $SEC = 1.12$. The prediction set for this calibration gave $r = 0.585$ and $SEP = 6.68$. In this case, the problem was due to noise in the sample spectra in the prediction set resulting from the high viscosity and colour of the syrup. This problem was corrected by diluting these samples and re-calibrating the application.

Regardless of all the statistical data comparing the calibration and the prediction sets, the true indication of whether or not the NIR analysis can replace the reference method is to compare the actual NIR data to the reference method data on as many samples as possible.

Operations Files for Routine Analysis:

Once the calibration is validated and tested, it is ready to be implemented. An operations file can be set up for the particular analysis which basically makes the NIR a "black box" and allows it to be used for routine analysis. Once established, only sample presentation to the NIR by an operator is required and analysis and display of data is handled by the operations file. An example of the output of an operations file for Jet Syrups is shown in Figure 4.

APPLICATIONS AND RESULTS

Figure 5 summarizes the data for the NIR applications that were investigated. The following are some examples of these NIR applications that are implemented and working at Redpath as well as some comments on other key applications that are being developed further for implementation. These applications give a good indication of how NIR can effectively be used as an alternative to conventional analyses.

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Remelt Syrups:

Calibrations were developed for Brix and POL in order to calculate purity for affination syrup and 1st crop syrups. Figures 6a and 6b show the typical calibration curves developed for this application. This analysis is done in 4 mm cuvettes and requires that the samples be diluted 1:1 with water. The development of this application originally started by analyzing the samples neat. However, predictions using this calibration were poor and this was found to be due to noise in the NIR spectra due to the physical attributes of the samples.

An interesting problem did occur during the start-up of this application in the Redpath control lab. Calculated purities from the NIR data were found to be consistently 3 to 4 units higher than the comparable lab method results. When the NIR spectra for these samples were examined, it was observed that the spectra were very noisy, similar to the problems observed with the un-diluted calibration set. Further investigation showed the noise to be the result of entrained air in the sample which was obtained from the refinery, diluted and analyzed immediately. This was not a problem during calibration and validation since the samples were allowed to sit (and de-aerate) before being analyzed on the NIR. The problem was resolved by de-aerating the samples in an ultrasonic bath just prior to analysis.

This application has significantly reduced the purity testing time for remelt samples and has also eliminated the use of the chemicals required for the conventional method. Examples of the typical results obtained with this NIR application are shown in Figure 7.

Liquid Invert Sugar:

Calibrations were developed to measure % invert in 50 grade liquid invert products. Figure 8 shows the calibration curve for this application. This is a relatively simple NIR analysis which is done on the neat sample in 4 mm cuvettes. This application is used to monitor the % invert on 50 Grade Invert during production of this product and the NIR application has significantly reduced the analysis time required with the conventional titration method. As well, the chemical requirements for the titration method have been significantly reduced since the NIR method has been implemented and the analysis can now be done by the liquid sugar operators when required. Figure 9 shows examples of some typical performance results using this application.

Process Liquors:

Calibrations for Brix, colour and ash were developed for Jet syrups and Filtered liquors and Fine Liquor. Initially, one calibration

set was developed for all of these product streams. However, predictions for colour were poor and required separate calibrations for Fine Liquor and the Jet/Filtered liquors. The following data for colour illustrates how the calibration for the Jet/Filtered liquor changed when the Fine Liquor samples were removed:

	<u>With Fine Liq.</u>	<u>Without Fine Liq.</u>
Calibration:	r = .994 SEC = 64.4	r = .997 SEC = 13.8
Prediction:	r = .698 SEP = 50.3	r = .994 SEP = 29.3

In this application, there still is opportunity for a universal calibration to cover all of these samples; however, many more samples will be required in the calibration set in order to make it reliable.

The ash calibrations for these liquors did not work well. This was also the case for most ash calibrations investigated during this evaluation. The area of the NIR spectrum which should be monitored for ash is not clear since this parameter is measuring inorganic constituents. In many cases, ash appeared to be related to absorbances in the visible end of the NIR spectrum (ie. higher colour - higher ash) however, this was not consistent. Further evaluations of the use of NIR analysis for measuring ash are required.

IMPLEMENTATION OF NIR IN THE PROCESS CONTROL LAB

The utilization of a working NIR system in the refinery process control lab at Redpath provided a good payback for the time and efforts spent on calibrating the system. NIR analysis of re-melt syrups and liquid invert products were chosen as the first applications to implement.

Since the lab employees already had hands-on experience with all of the regular conventional analyses, the minimal sample handling requirements for the NIR was not an issue. However, the lab employees had minimal experience with computers and, since the main operator interface with the NIR is via a computer, issues relating to training and generating and handling of NIR data were addressed.

As part of the training process, all control lab employees were first given a simplified description of the NIR and the process required for calibration. This was followed by detailed training on the use of the operations files for each particular analyses.

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The NSAS software is menu driven, but a separate flow diagram was prepared which enabled the operators to easily move around the menu as required. In order to prevent operators from using the wrong analysis, the operations files were named so that they could be easily related to the specific samples.

All operators were found to work well with the system after 2 to 3 days of working with it. Initially, the operators were required to double check their NIR analysis with the conventional methods in order to give them more confidence in the NIR capabilities and also to allow for more evaluations of the prediction performance of the calibrations. The NIR generated data is recorded on the main lab log as per the results from the conventional analyses and both a file and hard copy of all NIR generated analyses produced from the routine analyses are produced each day and stored for reference.

Maintenance of the NIR involves performing a monthly self diagnostic check using the NSAS software in order to monitor items such as lamp and detector conditions. All data files are backed up on a regular basis using a tape drive. The calibrations are checked weekly by running a minimum prediction set and comparing results with the original parameters for the calibration. These samples are also saved for expansion of the calibration set as required.

CONCLUSIONS

In this evaluation, NIRS was found to have many potential applications for analysis in the sugar refinery. NIRS is capable of providing a quick and simple method of analysis without reagents and with minimal sample handling requirements to support process control requirements. This provides a clear benefit to the refinery since operators can run many of their own analyses using NIR. Proper calibration of the NIR is important and requires time and effort as well as a good understanding of the calibration process. On-going development and implementation of the applications discussed in this report is continuing at Redpath with plans to evaluate calibrations produced with better reference methods and also to determine potential on-line applications.

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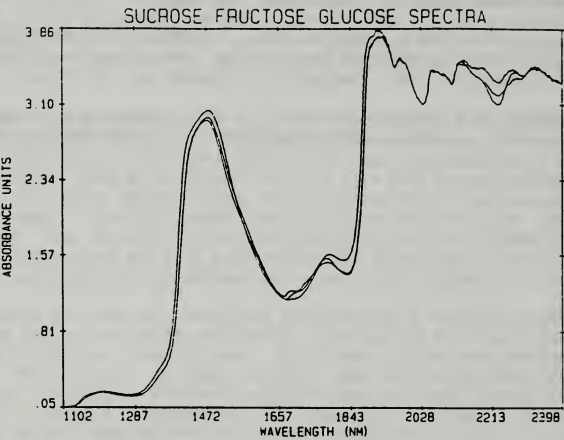


FIGURE 1

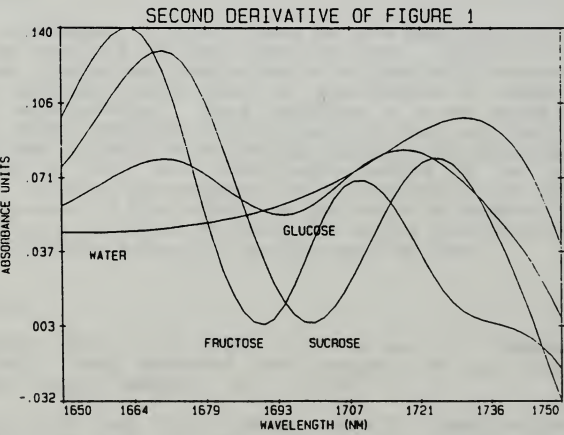


FIGURE 2

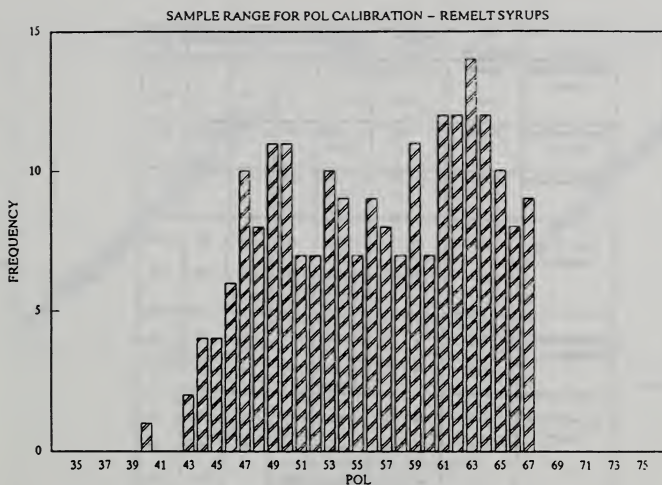


FIGURE 3

OPERATIONS FILE REPORT FOR ROUTINE ANALYSIS

Product: JETS-FILT

Date: Apr 25, 1994

Average of 4
Std Dev of 4

Grand Average 63.881 9.544
Grand Std Dev 1.771 2.158

Spl #	Spl Name	Time	BRIX	COLOUR x 100
-------	----------	------	------	-----------------

1	J2 BEF CHR	14:39:16	62.291	10.035
2	J2 AFT CHR	14:41:46	63.564	7.183
3	J3	14:44:39	65.789	11.414

FIGURE 4

SAMPLE	TEST PARAMETER	RANGE OF VALUES	CALIBRATION			PREDICTION	
			METHOD	r	SEC	r	SEP
White Granulated Sugar	Moisture	.010 - .040 %	1924 nm	.581	.004	.350	.006
	Colour	7 - 50 ICU	420 nm	.975	2.07	.910	3.25
Liquid Invert Sugar 50 Grade	% Invert	48 - 62 %	LS - 1680, 1752 nm	.859	1.17	.910	1.03
Liquid Total Invert	% Invert	82 - 97 %	LS - 1680, 1752 nm	.808	1.63	n/a	n/a
Remelt Syrup (diluted 1:1)	Brix	65 - 79	1742 nm	.987	.466	.981	.882
	POL	40 - 66	PLS 1650 - 1750 nm	.989	.926	.983	1.28
Remelt Syrup (no dilution)	Brix	65 - 79	1742 nm	.908	.863	.871	1.74
	POL	40 - 66	PLS 1650 - 1750 nm	.990	1.12	.585	6.68
Jet and Filtered Liquors	Brix	60 - 75	1366 nm	.991	.435	.993	.473
	Ash	0.1 - 2.00	1810 nm	.934	.161	.678	.255
	Colour	300 - 3500 ICU	678 nm	.991	.87	.937	167
Fine Liqueur	Brix	62 - 74	1738 nm	.998	.148	.988	.408
	Ash	.10 - .20	1794 nm	.796	.017	.784	.009
	Colour	95 - 300 ICU	522	.972	13.8	.935	24.8
1st and 3rd Crop Mass.	POL	60 - 85	PLS 1650 - 1750	.978	1.19	.960	1.54
	Invert	2 - 12 %	PLS 1650 - 1750	.977	.514	.938	.836
Soft Sugars	POL	85 - 95	PLS 1650 - 1750	.712	.591	n/a	n/a
	Invert	1 - 5 %	LS 1678, 1718 nm	.662	.217	n/a	n/a
	Colour	2500 - 12000 ICU	446 nm	.978	148	n/a	n/a
	Moisture	1 - 4 %	1960 nm	.956	.113	n/a	n/a
Molasses	Moisture	20 - 30 %	1156 nm	.890	.701	.910	.547

FIGURE 5. SUMMARY OF NIRS APPLICATIONS INVESTIGATED

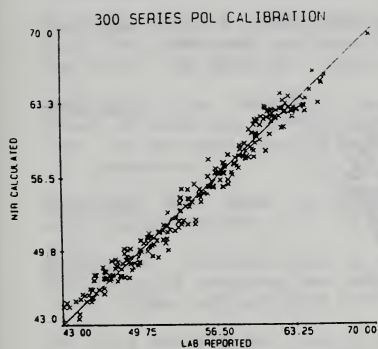


FIGURE 6(a)

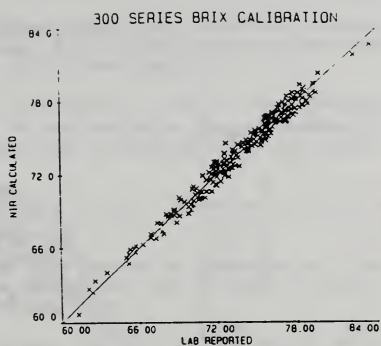


FIGURE 6(b)

LAB VS NIR CALCULATED PURITY
FOR REMELT SYRUPS

LAB PURITY	NIR PURITY
60.3	60.1
73.3	76.4
88.9	88.4
86.7	86.3
73.3	73.6
85.9	85.8
71.1	71.4
86.2	86.4
87	87.8
70.6	70.5
85.7	85.3
88.7	87.1
59.9	61.8
73.6	73.5
88.8	88.3
59.4	60.2
64.3	63.7
66.7	66.6
82.9	82.9
73.9	74.8

FIGURE 7

* samples monitored during week of Jan. 10/94 production

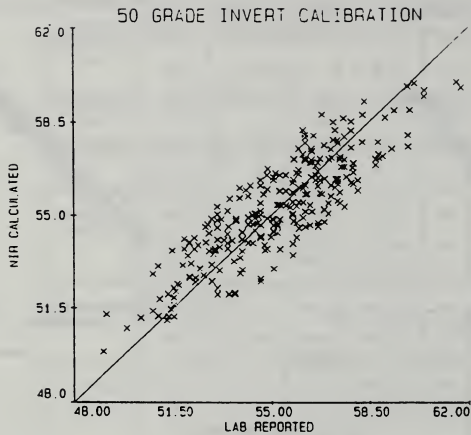


FIGURE 8

% INVERT FOR 50 GRADE LIQUID INVERT

LAB VALUE	NIR VALUE
57.37	56.63
56.67	56.96
57.62	57.95
56.43	56.38
51.97	52.94
52.40	52.87
51.90	52.77
54.25	53.84
54.02	53.89
58.74	58.57
58.91	58.63
56.35	55.99
49.75	50.56
55.75	54.91
47.26	47.92
51.25	51.95
50.02	50.72
55.60	54.96
41.70	42.37
50.39	49.97

FIGURE 9

* samples monitored during week of Jan 10/94 production

DISCUSSION

Question: That's great progress, that you've got operators running their own analyses. Do they actually bring their own samples into the lab? How does the operator run his own test?

Guglielmi: The instrument is in the Control Lab, on the process floor, beside the Control Room. We are making a common access to that lab, so that the NIR instrument can be used by both lab people and operators.

Question: What does the operator have to do? Does he call up a calibration curve?

Guglielmi: The analysis is set up for use as an Operations File. The operator presses the computer entry for the choice of Operations Files, and selects his Operation File.

The only problem we have at the moment is that we are still using quartz cuvettes (and breaking them routinely - too expensive), so we are looking at the use of probes in a fixed sample holder. We expect that to eliminate that problem - the basic cause is that we are currently using a lab instrument in a process environment.

Question: Thank you for the examples of real applications of this technology for your operators. I wonder if you would look into the future, on the basis of your experience so far, and indicate whether NIR will also have applications in your laboratory.

Guglielmi: NIR will be one of the main tools for process analyses, the control type testing, we require. It won't replace everything in the main laboratory, but the days of an operator bringing a process sample to the main lab for someone else to check, and then have the operator go back out and make an adjustment, are gone. This is where the NIR system will fit in. It won't obviously, replace every analysis, but will be a major tool available.

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THE USE OF NIR/NIT WITHIN THE FOOD AND AGRICULTURAL SECTOR

Leif Tilmanis

Tecator AB, Höganäs, Sweden

ABSTRACT

Applications of Near Infrared Reflectance (NIR) and Transmission (NIT) spectroscopy in food industries other than the sugar industries are presented. Products and constituents analysed are outlined, and development of calibrations described, for applications in the grain industry, the meat industry and the dairy industry.

INTRODUCTION

People have performed chemical analysis on materials for centuries, but only in the last 100 years has it become important to chemically analyse agricultural and food products. Analytical procedures for food and feed were developed in Europe in the late 1800's and spread to the United States and the rest of the world by the early 1900's.

One of the first analysis schemes, called proximate analysis, evaluated a sample of material for crude protein, fat, crude fibre and moisture. Subtracting the total of these substances from 100 yielded a value that included all other non nitrogen compounds in the sample. This value was called nitrogen-free extract. By the late 1930's, this analytical scheme was used by nutritionists to establish feed requirements for animals. Scientists used it to learn more about the structure and function of plants and animals. Food scientists used it to establish the composition of new products. Proximate analysis became the accepted standard for analysis in the food and agriculture industry and is still used today.

As the chemistry of plant and animal products became better understood, scientists found that proximate analysis provided only a crude analysis of a material. More sophisticated analytical schemes were developed to further characterize the complex chemistry, leading to hundreds of new analytical methods and the foundation of the American Association of Official Analytical Chemists (AOAC), in 1886 and similar organizations. At the same time, manufacturers refined the laboratory equipment used in these

analyses and researchers sought ways to make this new information more accessible.

By the late 1950's, analytical techniques had been refined to the point where materials could be analysed for hundreds of individual compounds. Universities began to establish whole departments devoted to conducting analyses.

Today, libraries are filled with detailed information on analytical procedures and laboratories are filled with the equipment to provide this information. Plant breeders, nutritionists, people who buy and sell agricultural goods, food engineers and so on need this information, but the costs and timeliness of analysis are consistently limiting factors.

If the physical and chemical composition of a sample could be analysed in seconds, with little or no sample preparation, many analytical problems would be solved. During the 1960's the first commercial NIR instruments were developed, using a small segment of the electromagnetic spectrum called near infrared. This small segment begins above the visible region, at 700 nm, and continues to the infrared region, at 2500 nm.

The first measurement where NIR was applied was moisture in wheat. Today the technique covers a vast number of analyses and companies like Tecator AB and NIRSystems can supply precalibrated instruments. NIR/NIT analysis offers many advantages like speed, nondestructive sampling and environmental safety. It requires little or no sample preparation and multiple constituents can be analysed simultaneously. Even though NIR/NIT does measure many constituents with a high degree of accuracy, it cannot measure everything. The functional groups that respond to NIR/NIT radiation are hydrogen (-H) bonds. NIR/NIT analysis is and remains a secondary method of measurement and the instruments must be calibrated with the primary laboratory methods.

CALIBRATION PROCEDURES

Many factors affect the accuracy of a secondary method such as NIR/NIT. If the reference method is inaccurate the accuracy of the secondary method might be reduced. Grouping of samples into products also affects the accuracy. To get the highest accuracy one would need to separate samples based on variety, sample processing, maturity and so on. However, this would form too many products which would make it difficult to correctly identify new samples. The solution is to combine groups with similar spectra - to group sample chemistry relationships into products that can be easily distinguished. For example, grains are divided into

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products by plant species such as wheat, corn and barley. When new samples are analysed the accuracy of NIR/NIT analyses can be reduced if they are not represented by the calibration samples. Calibration sets often must contain samples with special features to stabilize the calibration. Such samples could be samples with temperature above or below normal temperature, samples from an unusual growing season and so on. The calibration's constituent range should cover most of the values expected in routine analyses.

After ensuring that the samples selected represent the product, a second selection is done in most situations. Normally a large number of samples are available, but obtaining laboratory reference values for all of them would be very expensive. Therefore samples for calibration are selected on the basis of their spectral information using the principal components to condense the information in a full spectra into a smaller set of scores. This information is then used in selecting a population where outliers and neighbors have been removed as these, in most cases, will not contribute to the calibration's accuracy. Samples with extreme spectra should be retained if they are valid extensions to the population. Including them in the calibration broadens the range of samples it can predict.

When the calibration population has been selected and effects associated with interfering absorptions have been minimized (by applying scatter correction and/or working with derivatized data) the appropriate calibration method needs to be applied. There are a number of different methods but two of the most common used are Multiple Linear Regression (MLR), usually used in simple compositions where unique spectral features are identifiable, and Partial Least Squares (PLS), usually used in complex compositions where unique spectral features are not readily identified or absorption bands are susceptible to matrix effects.

Once an NIR/NIT equation has been developed the accuracy should be verified, most commonly by using the Standard Error of Prediction (SEP) or Standard Error of Cross Validation (SECV) statistical values as a measure for the performance. The SEP is obtained by predicting a representative test set of samples not included in the calibration set. The SECV is obtained by predicting samples removed from the calibration set; different samples are removed each time until all samples have been predicted once. Each time samples are removed the remaining samples are used for calibration, therefore the predicted samples are not included in the calibration. Also the proportion of explained variance (r^2) or the correlation (r) will show the strength of the relationship between the primary reference method and the NIR/NIT analysis.

PRECALIBRATED INSTRUMENTS

Precalibrated instruments, like NIRSystems instruments and Infratec from Tecator AB, offers many advantages and can usually be used for analysis the same day as received. However NIR/NIT is a secondary analytical procedure and precalibrated instruments generally are limited to samples with a common spectra-sample chemistry relationship. The sample must be presented to the NIR/NIT instrument in the same manner as during calibration development. The accuracy of NIR/NIT analysis should be evaluated with the equation statistics provided with the precalibrated instrument. The range should cover most values expected in routine analysis. There are software tools, as in the NIRS software from Infrasoft International (ISI), to easily expand the product calibrations with new samples to eliminate problems like this.

Companies like ISI also offer commercially available calibration equations for a large number of food and agricultural products like forages, feeds and feed ingredients. An example of some of these can be seen in Table 1, 2, 3 and 4 (2). These calibrations were developed from extensive food and agricultural spectral libraries. These spectral libraries were formed over a period of years with samples collected from many locations throughout North America and other parts of the world. Reference values were then obtained from certified laboratories for the calibration samples. Calibration equations were developed using a modified partial least squares (PLS) regression technique. Instruments from NIRSystems were used.

In the precalibrated Infratec Meat Analyzer 1265 (NIT), general meat calibrations that cover most kinds of ground meat from beef and pork are included. Variations covered is hot/cold boning-shoulder, belly, loin, leg, neck, ribs, etc.- salted/unsalted-additives like spices, carbohydrates, protein etc.- frozen, fresh and defrosted, see Table 5. For example, the MM1 calibration is based on a calibration set of 80 samples originating from different countries. The test set used for verifying the accuracy (SEP) contains 482 samples (none of them included in the calibration set). Calibration equations were developed using a partial least squares (PLS) regression technique. These general calibrations, which are very robust, have the advantage that most ground meat samples can be analysed. The disadvantage with broad calibrations like this is the reduced accuracy. This means that local calibrations, or calibrations optimized with local samples, are preferred due to their higher accuracy (lower SEP), see Table 6. The support of local calibrations is done by Tecator AB, free of charge, after receipt of spectral data and reference data for fat, protein and moisture. Other products where applications have been done are for different sausages, different type of final products like paté and cold cuts and also for chicken.

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AT-LINE AND ON-LINE MEASUREMENTS

The second largest food based market after the meat market in milk and dairy processing. Within milk and dairy processing there are a number of on-line installations (NIRSystems instruments) as well as at line installations (NIRSystems instruments and Tecator AB Infratec Food & Feed Analyzer 1255) for measurement of different products, covering the range from raw milk through processing to final products like cheese, yoghurt and ice cream. Depending on product, both reflectance and transmission measurements are being used. Typical parameters predicted are protein/casein, fat, moisture, solids and lactose, as these parameters are governed by product specification and regulation.

In the production of dairy products, compositional analysis of milk is performed to ensure that the milk meets minimum nutritional requirements and it also serves as the basis for standardization of the milk prior to processing. In a preliminary application study for one customer, a calibration for fat in raw milk was made. An NIRSystems Enhanced Dairy Analyzer, with sample transport module measuring in transmission with 1mm cuvette, was used for the measurements. Fifty samples were scanned, thermostatted to 25°C, these covering most of the variations that will occur such as samples directly from tank-cars, storage tanks, diluted samples and some seasonal variation. None of the samples were homogenized and reference values were obtained as the average of duplicates from the Rose Gottlieb method. From the scanned samples, 8 of them were removed randomly as an independent test set (the low number due to the limited number of scanned samples). From the remaining samples a PLS calibration equation was developed over the 700-1100 nm wavelength range, using the NIR3 routine operation, calibration development and network management software package from Infracore International. This giving a calibration over the range 2.33 - 7.68% fat with a standard error of cross validation (SECV) of 0.13, see Table 7. In Figure 1 the calibration curve for fat can be seen. The calibration equation developed was evaluated with the test set giving a standard error of prediction (SEP) of 0.09%, see Table 8.

An application study on creamery butter in the process has also shown that calibrations developed on laboratory instruments can successfully be transferred to the on-line system (3). NIRSystems Process Analytics on-line analyzers use fibre optics to interface directly into the process stream, providing real-time analysis and control of the manufacturing process.

ACKNOWLEDGMENTS

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2. ISI, NIRSystems and Tecator AB. (1994). NIR calibration equations, for food and agricultural products for use with ISI software.
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Table 1
PC-0904 MAY AND FRESH FORAGE
IC-0904 (INTERNATIONAL NUMBER)

Total samples evaluated = 1999

Samples were selected from US, Canada, and Europe. They include pure species and mixtures.

Variable	N	RANGE	Mean	SECV	RSQ
PROTEIN	1214	1.85 - 32.10	17.03	0.83	0.86
ADF	1214	1.85 - 32.10	35.02	1.70	0.83
ADP	1214	0.01 - 13.40	0.89	0.20	0.90
CA	1188	0.08 - 0.84	0.31	0.08	0.94
K	1211	0.11 - 3.02	1.04	0.16	0.84
MG	1211	0.27 - 8.31	2.27	0.48	0.55
DM	1228	84.10 - 89.88	82.39	0.73	0.85
NDP	550	29.70 - 84.10	54.42	2.73	0.84

Samples were field cured, microwave, or oven dried at 60°C and cyclone mill ground. N = number of samples selected by SELECT for calibration.

Table 2
PC-0813 WHEAT
IC-0813 (INTERNATIONAL NUMBER)

Total samples evaluated = 845

Samples were selected from US and Canada. All wheat types are included in this calibration.

Variable	N	RANGE	Mean	SECV	RSQ
PROTEIN	287	8.15 - 24.20	14.82	0.42	0.88
ADF	287	2.15 - 12.00	3.80	0.38	0.73
DM	283	88.51 - 92.00	89.17	0.38	0.87

Samples were not dried, but cyclone mill ground. N = number of samples selected by SELECT for calibration.

Table 3
PC-0909 SOYBEAN MEAL
IC-0909 (INTERNATIONAL NUMBER)
Total samples evaluated = 704

Samples were selected from US and Canada.

Variable	N	RANGE	Mean	SECV	RSQ
PROTEIN	229	46.64 - 58.06	54.71	0.81	0.81
ADF	220	0.33 - 4.48	1.37	0.17	0.90
CF	174	1.83 - 10.22	4.52	0.47	0.81
DM	232	86.84 - 92.15	89.50	0.57	0.75

Samples were not dried, but cyclone mill ground. N = number of samples selected by SELECT for calibration.

Table 4

PC-0820 FISH MEAL
IC-0820 (INTERNATIONAL NUMBER)

Total samples evaluated = 252

Samples were selected from US and Canada.

Variable	N	RANGE	Mean	SECV	RSQ
PROTEIN	148	53.90 - 80.70	68.04	1.33	0.87
ADF	150	1.87 - 17.52	8.37	0.58	0.89
DM	148	84.80 - 95.20	81.83	0.35	0.97

Samples were not dried or ground, but spectra from 2 subsamples averaged. N = number of samples selected by SELECT for calibration.

Table 5

Universal Ground Meat (Minced Meat) Calibrations

Cal.ID	MM1	MM2	MM3
Range of fat	1 - 10 %	5 - 18 %	15 - 30 %
SEP			
fat	0.35	0.54	0.62
moisture	0.55	0.57	0.56
protein	0.47	0.49	0.43
collagen	0.6	0.6	0.6
Cal.ID	MM4	MM5	
Range of fat	25 - 40 %	35 - 55 %	
SEP			
fat	0.47	0.69	
moisture	0.43	0.55	
protein	0.31	0.41	
collagen	0.6	0.6	

Products: Ground beef and pork originating from USA, Germany, Sweden, Finland, Denmark and Switzerland. The samples have been selected from many different parts of the animals (head, shoulder, belly, loin, tenderloin, neck, leg, ribs, rump, brisket, thick flank, etc.).

Table 6

APPLICATION REFERENCES

Ground Meat			
	Hero, Switzerland	Mariensee Germany	
Cal.ID	CH0518	RI1	SW1
Range of fat	1.5-19.4	0.3-5.8	0.6-2.9
SEP			
fat	0.54	0.22	0.09
moisture	0.62	0.22	0.16
protein	0.49		
Products:	Veal (Kalb) Pork (Schwein) Beef (Rind) "Grundbrät mit Gewürz und Salz" "Fertig roh Brät" "Grundbrät mit Gewürz und Mioloheweiz"	Beef (Rind)	Pork (Schwein)
	Scanfood, Sweden	Danish Meat Research Inst.	
Cal.ID	ST1	BOV	KAM
Range of fat	11.5-23.2	2.5-8.1	0.8-5.0
SEP			
fat	0.46	0.24	0.18
moisture	0.50	0.47	0.46
protein		0.24	0.29
Products:	Cooked pork and beef patties	Pork, shoulder	Pork, fore leg

Table 7

Filename: MILK.EQA
Thu Aug 04 13:40:38 1994

Raw milk, 2.33 - 7.68% fat.

Variable	N	Mean	SEC	RSQ	SECV	1-VR	#
1:FAT	41	4.43	0.08	1.00	0.13	0.99	196 1

Table 8

Filename: MILK TST.CAL vs MILK.EQA
Thu Aug 04 13:35:08 1994

	Pair 1	
	FAT	FAT
SEP		0.09
Means	3.82	3.85
BIAS		-0.03
BIAS Limit		0.05
SEP(C)		0.09
SEP(C) Limit		0.11
Stand Devs	0.73	0.75
slope		0.97
RSQ		0.98
Average H		0.57
N		8

Pair 1					
FAT vs FAT					
Samp number	LAB	ANL	Residual	Residual-BIAS	'H'2
931206-3	4.42	4.45	-0.03	-0.00	0.19
931208-4	4.28	4.32	-0.04	-0.01	0.07
931207-3	2.82	2.78	0.04	0.07	0.77
940302-7	3.71	3.57	0.14	0.17	0.45
931111-13	4.91	4.92	-0.01	0.02	0.50
940223-4	3.47	3.62	-0.15	-0.12	0.55
931111-11	4.03	4.18	-0.15	-0.12	1.41
940223-1	2.91	2.95	-0.04	-0.00	0.60

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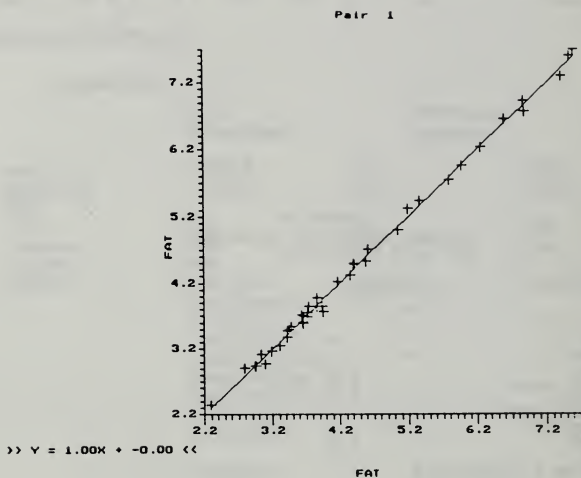


Figure 1

DISCUSSION

Comment: Mr. Tilmanis did not have time to mention that, in addition to meat products, several grains, grain product, and dairy industry products are currently bought and sold on Near Infrared analysis.

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**NEW UTILIZATION OF THE NIR TECHNIQUE: CONTROL OF AN ION EXCHANGE
RESINS SYSTEM FOR LACTIC ACID RECOVERY FROM FERMENTATION.**

Giuseppe Vaccari, Elisabetta Dosi, Anna Lisa Campi and
Giorgio Mantovani

University of Ferrara, Ferrara, Italy

ABSTRACT

At the last meeting of S.P.R.I., held in New Orleans in 1992, a proposal for the on-line utilization of the NIR technique to control fermentations was presented and discussed. The metabolite recovery is carried out by using an innovative ion exchange resins system. The optimization of such a downstream process can be achieved by utilizing the NIR technique on-line. The results obtained are presented and discussed, emphasizing the possibility of applying the NIR control to ion exchange resins in general.

INTRODUCTION

The fields of application of the NIR technique are becoming increasingly numerous. In order to realize this fact it is sufficient to consider the papers presented at the last International Conference on NearInfrared Spectroscopy recently held in Australia (4).

Furthermore, within each field of application both the materials to be analyzed and the parameters to be evaluated via the NIR technique are increasing substantially. On-line analysis using NIR is no longer a problem for many applications and automated plants can already be managed using the NIR data recorded in real time.

The sugar industry began to study applications of the NIR technique in the second half of the 1980's. Since then interest in this analytical methodology has soared. It is now very difficult to cite the many papers published on this subject. In order to get an idea about this area it is sufficient to peruse the Reports of the various Referees recently presented at the 21st Session of ICUMSA in Havana (5). Moreover, the recent paper of Schäffler et al. (6) presented at the 1993 SASTA Congress gives an overview of the most significant literature which has contributed to the spread of the NIR technique in the sugar area.

Biotechnology is a sector where more recently the great advantages of the NIR technique have been appreciated, in particular with

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regard to the control of fermentation processes (1,3,7,9). The recording and relevant utilization of the NIR data from a computerized unit managing a fermentation process is becoming real, according to the hypothesis which we formulated at the last SPRI congress in New Orleans in 1992. However, each fermentation process must be followed by the recovery of the product or the products of the fermentation itself, so why not utilize the NIR technique for the control of this downstream process?

We describe below an innovative process for the recovery of the lactic acid from fermentation broths using ion exchange resins (2,8), pointing out the possibility of utilizing the NIR technique to follow and optimize the process.

EXPERIMENTAL AND RESULTS

The classical scheme for the production and recovery of lactic acid from fermentation broths (Figure 1) has various disadvantages which can be summarized as follows:

- discontinuous fermentation
- low concentration of lactic acid
- low conversion rate
- impossibility of biomass recovery and recycling
- problems of by-products disposal
- low purity of the lactic acid produced.

In order to solve such problems we have studied an innovative system which, following a micro-filtration module for biomass separation and recycling, utilizes an ion exchange resin system for the recovery and purification of lactic acid (2,8). Table 1 gives the broth composition and the type of microorganism utilized.

The basic concept of the lactic acid recovery system may be obtained from studying Figure 2. The broth with cells removed leaving the micro-filtration plant flows through a strong anion exchange resin bed (Amberlite IRA-420, Rohm and Haas, USA) in the carbonate form. The lactic acid fixed by the resin is then eluted using a solution of ammonium carbonate and this automatically regenerates the resin. The latter can then be directly reutilized after washing. The eluate from the resin is pre-concentrated by boiling, at the same time recovering the ammonium carbonate. The residual solution of ammonium lactate is treated with a strong cationic resin in the hydrogen form (Amberlite IR-120, Rohm and Haas, USA) in order to obtain the lactic acid, which is finally concentrated. Whilst in New Orleans (5) we described the utilization of NIR for fermenter control, proposing the direct interfacing between NIR and the computer of the fermentation process as shown in Figure 3. We will discuss now the utilization of NIR for the

control and the management of the lactic acid recovery system from broth using anionic resins.

The various steps of the broth treatment with the anionic resin are shown in Figure 4. In step 1 the broth with cells removed flows through the anionic resin; the amount of broth depends upon both its lactic acid concentration and the resin exchange capacity, which latter progressively decreases with the increase in the number of cycles. This decrease is due to the fact that strong anions, such as sulfate (see Table 1), which are present, albeit at very low levels, are fixed by the resin without later being eluted with the ammonium carbonate solution. The only way to decide to interrupt the treatment of the resin with broth is to know in real time the concentration of lactic acid leaving the resin itself. By this interruption, three goals are achieved i.e.: 1) the exchange capacity of the resin is utilized at the maximum level; 2) excessive amounts of lactic acid are not recirculated to the fermenter; 3) it is possible to decide when the resin has to be completely regenerated with sodium hydroxide, before being put into the carbonate form again. Bearing in mind that, (see Figure 2), the broth leaving the anionic resin, after addition of the substrate, is reintroduced into the fermenter, it is important to know the glucose concentration in the effluent and then to evaluate the amount of glucose to be added. Likewise it is important to be able to follow the concentration of lactic acid during step 2 in order to know the minimum required amount of washing water. In step 4 it is important to know the minimum amount of ammonium carbonate to be added. Thus, an insufficient amount of the latter would leave some lactic acid on the resin, whereas an excessive amount would involve a waste of this regenerant, a dilution of the ammonium lactate solution and wasted energy as a consequence in the concentration step. Also in this case it is important to know, in real time, the variation in the lactate concentration leaving the resin.

Calibration curves have been prepared and suitable validation tests carried out to determine the concentrations of lactic acid (metabolite) and glucose (substrate) leaving the column during the treatment with broth and the washing with water (steps 1 and 2), and for determination of the lactate ion concentration during the elution with ammonium carbonate (step 4). Subsequent cycles of treatment of the broth with the same resin were checked using the curves obtained.

A Bran+Luebbe INFRAALYZER 450 equipped with 19 filters and fitted with a cell for liquids, was employed.

The reference analyses of the various samples for the calibration and validation tests were carried out by HPLC. An Aminex column

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HPX-87H, 300x7.8 mm, temperature 25°C, 0.005 mol/l H₂SO₄ as eluant, 0.6 ml/min flow, were adopted and a refractive index detector employed which permitted simultaneous determination of glucose and lactic acid.

Control of the resin effluent in steps 1 and 2.

Forty samples coming from various cycles of treatment of the fermented broth with anionic resin and taken from steps 1 and 2, were utilized for the preparation of the calibration curves. As usual we selected the samples in such a way as to include, as uniformly as possible, the whole range of concentrations found for both lactic acid and glucose. The samples were read three times in the NIR equipment which then recorded the absorption data corresponding to the 19 wavelengths of the filters. The best calibration curves were obtained using two filters for lactic acid and 6 filters for glucose. Figures 5 and 6 show the calibration curves obtained for lactic acid and glucose, respectively. Another 31 samples of effluent from the anionic resin were used for the validation test. Such samples were read three times in the NIR equipment, after HPLC analysis, using the calibration curves previously plotted, and the data of the three readings were averaged. Figures 7 and 8 show the results of the validation tests which confirm the reliability of the plotted calibration curves. Using such calibration curves it was possible to continuously follow the composition variation of the effluent from the anionic resin during repetitive treatment cycles, using the same type of broth obtained via batch fermentation having a lactic acid concentration of approximately 8 g/100 ml. Figure 9 shows the comparison between the behaviour of the resin during its first cycle and the eleventh cycle. The first parts of the curves (closed symbols) correspond to step 1 whilst the second parts (open symbols) correspond to step 2, i.e. the resin washing. The higher efficiency of the resin during its first cycle in comparison with the eleventh cycle is clear. Obviously, having such curves available in real time makes it possible to decide when it is necessary to completely regenerate the resin with sodium hydroxide, before starting another series of repetitive cycles in the carbonate form. Moreover, from the data which are given in sequence by the NIR in real time, we can decide how long to continue step 1 before starting step 2 of washing and how long to go on with this step before changing to the next step. The glucose concentrations in the various samples shown in Figure 9 were approximately zero because we used broths from batch fermentations where all the substrate had been metabolized. Obviously, if the fermentation had been carried out another way, i.e. a continuous fermentation, the residual glucose concentration could not have been zero. In such a case, knowing the glucose concentration in the effluent from the

column, it would be possible to calculate the amount of additional substrate to be added before recycling the broth to the fermenter.

Control of the resin effluent during step 4.

In order to follow the variation of the lactate ion concentration in the resin effluent during the ammonium carbonate recovery step, we had to prepare a new calibration curve because the matrix was completely different in comparison with the samples from steps 1 and 2. We utilized 41 samples originating from different stages of the resin elution. Also in this case the samples were read three times in the NIR equipment. The best calibration was obtained using four filters and the relevant results are given in Figure 10.

The high value of the correlation coefficient can be explained because the matrix effect is very low with the solution containing practically only ammonium lactate and ammonium carbonate. The validation was carried out using 31 samples read three times in the NIR equipment; the relevant results were averaged. The data shown in Figure 11 guarantee the reliability of the calibration curve obtained. Using such a curve various cycles of lactic acid recovery were followed according to the methodology described in the previous section. Figure 12 shows the results obtained for ammonium lactate elution during the 1st and the 11th cycles of utilization of the resin as was the case in Figure 9. Also on the basis of such data the loss of efficiency of the column due to the increasing of the number of the treatment cycles is clear. The possibility of following in real time the variation of the lactate ion concentration certainly guarantees optimization of the elution. Advantages include avoidance of the utilization of a wasted excess of eluant, the occurrence of a certain amount of lactic acid being left on the resin, and collection of the eluant when the lactic acid concentration is judged to be reasonable.

CONCLUSIONS

We believe that the procedure described above makes it possible to assess how important it can be to know, in real time, what is occurring from the chemical point of view during the treatment of a solution with a resin plant. By now the completely automated resin plants are managed on the basis of evaluations made *a priori*, starting from the assumption that some operational parameters are adopted, such as e.g. steady solution composition, the maintenance of a certain flow, the steady exchange capacity, optimum regeneration conditions, and so on. The possibility of following, from a chemical point of view and in real time, the development of the various process steps could allow better utilization of the plant, in particular from the economic point of view.

ACKNOWLEDGEMENTS

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Table 1. Broth composition

Microorganism	<i>Lactobacillus casei</i> DSM 20001
pH	6.5
Temperature	37°C
Anaerobic or microaerobic conditions	
Maximum glucose concentration	10%
Yeast extract	3%
MgSO ₄ · 7H ₂ O	0.06%
FeSO ₄ · 7H ₂ O	0.003%
MnSO ₄ · H ₂ O	0.003%
Sodium acetate	0.1%
KH ₂ PO ₄	0.05%
K ₂ HPO ₄	0.05%
Maximum lactic acid concentration	8 - 10%

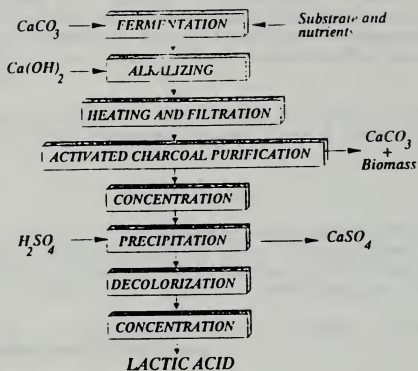


Figure 1 - Classical scheme of lactic acid recovery from fermentation broth

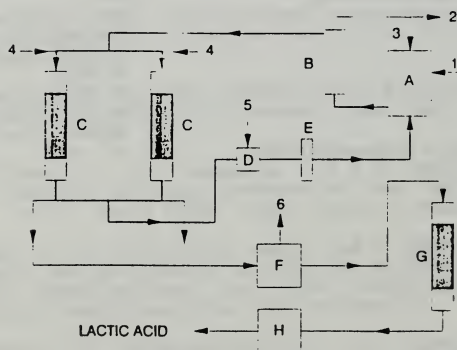


Figure 2 - Innovative scheme for lactic acid recovery from fermentation broths using an ion exchange resin system (8, 9) A) continuous fermenter, B) micro-filtration module, C) anionic resin columns, D) collection of the treated broth, E) sterilizing filter, F) pre-concentration, G) cationic resin column, H) final concentration; 1) additives; 2) biomass recovery, 3) biomass recycle, 4) lactic acid elution; 5) nutrients addition; 6) ammonia and carbon dioxide recovery

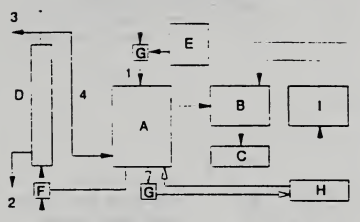


Figure 3 - Interfacing of the NIR and the fermenter computer: A) fermenter; B) computer; C) recorder; D) micro-filtration; E) feed supply; F) pump; G) peristaltic pump; H) measurement cell; I) NIR; 1) feeding; 2) micro-filtered broth; 3) biomass blowdown; 4) recycling.

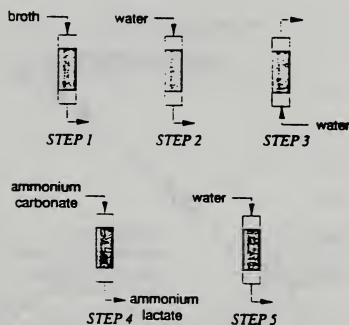


Figure 4 - Summary scheme of the various steps of the broth treatment on anionic resin. Step 1: percolation of the fermented broth; Step 2: co-current washing with water; Step 3: counter-current washing with water; Step 4: elution with ammonium carbonate; Step 5: co-current washing with water.

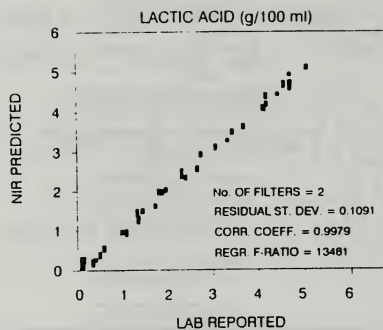


Figure 5 - Calibration curve for lactic acid.

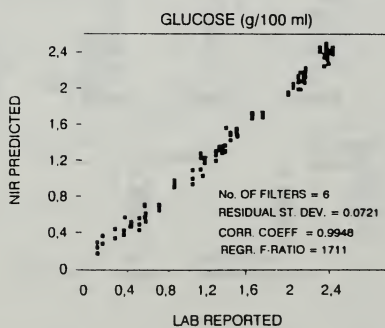


Figure 6 - Calibration curve for glucose

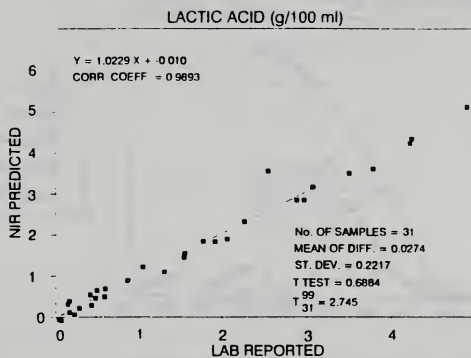


Figure 7 - Validation curve for lactic acid.

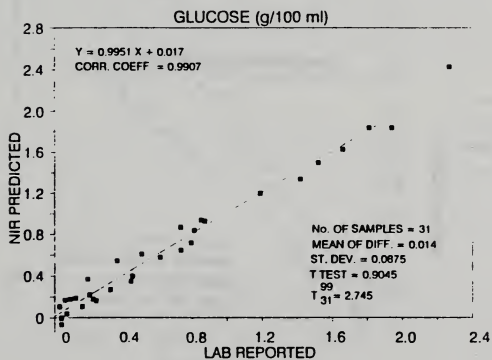
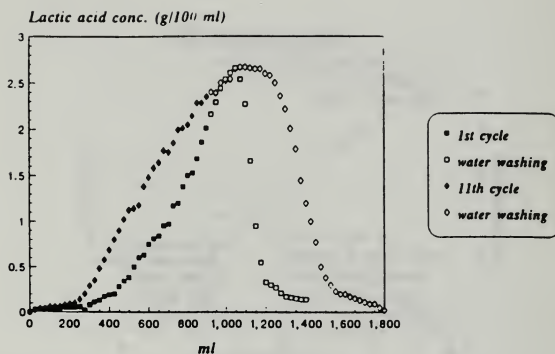


Figure 8 - Validation curve for glucose.

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(Lactic acid concentration in the broth entering = 8 g/100 ml)

Figure 9 - Lactic acid concentrations in the effluents from the anionic column during steps 1 and 2, recorded by the NIR on-line during the 1st and 11th working cycles of the resin.

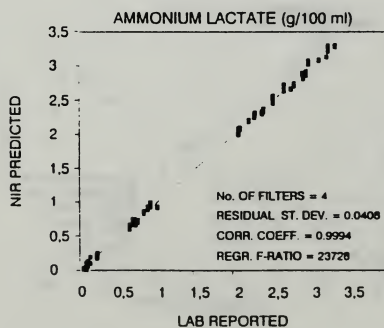


Figure 10 - Calibration curve for ammonium lactate.

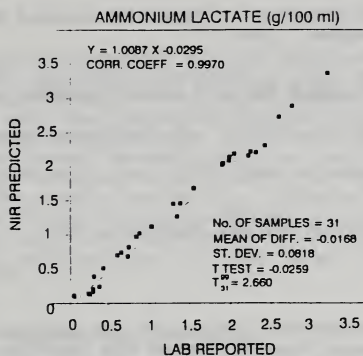


Figure 11 - Validation curve for ammonium lactate

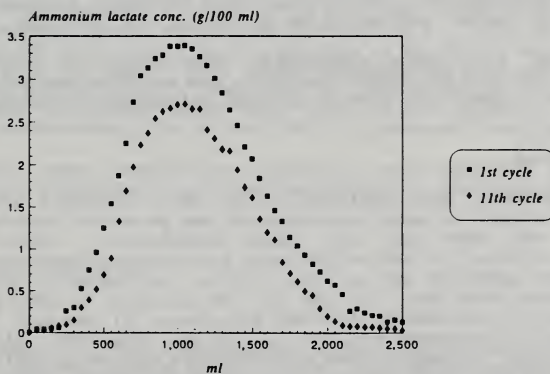


Figure 12 - Ammonium lactate concentration variations in the effluents leaving the anionic column during step 4, recorded by the NIR on-line during the 1st and 11th working cycles of the resin

UNIVERSAL NIR CALIBRATIONS FOR THE SUGAR INDUSTRY

Les A. Edye¹, Margaret A. Clarke¹ and Cynthia McDonald-Lewis²

¹Sugar Processing Research Institute, Inc., New Orleans, Louisiana
USA 70124

²NIRSystems, Inc., Silver Spring, Maryland, USA

ABSTRACT

Over the past four sugarcane crops and the past three sugarbeet-crops, SPRI researchers have collected NIR spectra of raw materials, process streams, and products (and conventional analytical data) from several cane and beet factories. The primary objective of this data collection has been the development of universal NIR calibrations that accommodate all seasonal and geographic variations in the quality of the raw materials (sugarcane and beets) and the resulting process streams and products. While in many cases the result of this exercise has been calibration of the NIR spectrophotometer for enduring reliable analyses, in some instances reliable universal calibrations are still not possible.

This paper highlights successful completion of universal NIR calibrations for the sugar industry, and address obstacles that in some cases hinder the development of universal calibrations.

INTRODUCTION

The possibility for application of near infrared analysis to process control in sugar manufacture has prompted several studies in both the beet and cane sugar industries. Reported sugar industry studies include reflectance and transmittance spectroscopy using both scanning and multiple filter instruments. Reflectance spectroscopy has been applied to the analysis of whole shredded sugarcane (1) and whole fibrated sugarcane (2) for pol (viz., the measurement of sucrose content by optical rotation), fibre, sugar and moisture. Transmittance spectroscopy has been applied to the analysis of sugarcane juice (3, 4), and diffusion, thin and thick beet juices (5, 6) for pol and refractometric dissolved solids. These analyses of major components in the sample matrix have generated results with errors similar to, or slightly greater than, the errors of conventional analytical methods. The advantage of near infrared multicomponent analysis over conventional methods is in time and labor savings.

In general, the development of new applications has been more successful with scanning instruments than with multiple filter instruments. Furthermore, advances in chemometric analysis methods have increased both the potential applications and the sensitivity of near infrared spectroscopy. With this perspective, we have successfully broadened the application of near infrared spectroscopy in the sugar manufacturing industry to the analysis of minor components in the sample matrix (i.e., glucose and fructose in cane and beet juices (7, 8), and polysaccharides in raw cane sugar (9)). This paper describes some highlights in the ongoing development of universal NIR calibrations at the Sugar Processing Research Institute (SPRI), Inc.

EXPERIMENTAL - METHODS AND MATERIALS

Reflectance and transmittance spectra (400 to 2500 nm) were obtained using an NIRSystems 6500 scanning NIR spectrophotometer (NIRSystems, Inc.). For reflectance spectra, crystalline raw sugar samples were packed into a sample cup with a circular face (5 cm dia., 0.5 cm depth), beet brei was spooned into a quartz cuvette (3 cm depth), fibrous cane material from core samplers and finer ground cane from a Jeffco cutter-grinder were placed in a coarse cell (rectangular quartz face, 3.5 x 16.5 cm). For transmittance spectra, aqueous sugar processing streams (i.e., cane juice, beet thin and thick juice) in a quartz cuvette (1 mm depth) were scanned 50 times and averaged. The sugar processing streams were not filtered or clarified with chemicals or pretreated in any way. The spectra were analyzed using NSAS (Ver. 3.25) spectral analysis software (NIRSystems, Inc.) and NIRS 3 (Infrasoft International).

The various sugar containing materials were analyzed using the conventional methods of the sugar manufacturing industry (e.g., 10, 11).

RESULTS AND DISCUSSION

General

The progression of NIR application development is shown in Figure 1 and the current status of the Sugar Processing Research Institute's development of various sugar industry NIR applications are shown in Tables 1 to 3.

Universal calibrations for beet diffusion and thin juice

Details of the developed universal calibrations for the prediction of pol and Brix in beet diffusion and thin juices are shown in Table 4. It is truly remarkable that the calibration models for

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prediction of pol in beet diffusion and thin juices are based on linear regression at the same single wavelength. Furthermore, the spectral data for diffusion juice and thin juice samples can be combined into a single data base that can be used to create a satisfactory model for prediction of pol in either of the process streams. In all cases the calibration models for prediction of Brix values in these streams are based on multiple linear regression at the same two wavelengths ("sucrose by pol" wavelength + offset wavelength to account for other dissolved solids). The physical basis for these models is shown by the absorbance of pure sucrose solutions in Figure 2. At these wavelengths the amplitudes of the second derivative spectra of the pure sucrose solutions are proportional to sucrose concentrations.

A plot of NIR results versus polarimetric results for the universal calibration for pol in diffusion and thin juices is shown in Figure 3. The calibration was developed from the data of six factories in one year with return visits to three factories the following year. The calibration fit for thin juice data from four of the factories in one year is shown in Figure 4. The conformance of factories A, B and C to the slope of the calibration indicates that a major source of error is instrumental bias among factories (i.e., the conventional polarimetric data was obtained using the factories' instruments, and variation in instrument calibration accounts for much of the error). In the case of factory D, the data range obtained from the NIR prediction of pol is 60 % greater than that obtained by the conventional measurement. It seems most likely that either the polarimeter at factory D was faulty, or the lower pol values were not reported correctly. On a subsequent visit to this factory the thin juice data conformed to the universal calibration.

While the beet diffusion and thin juice pol universal calibration standard error of 0.44 % w/v may appear to be too great for useful application, the calibration range, from 5.8 to 15.0 % w/v, covers all likely variation in juice quality. The calibration data base is a useful starting point to which individual users can continue to contribute their own factory data. In this process the standard error will decrease, and the user can be confident that the calibration will produce reliable pol readings over a wide range in juice quality (a range that may not be encountered in a single factory under normal operating conditions in any one crop year). As an example, juice pol data and NIR spectra obtained from a beet factory in the mid-western USA on a visit in 1992 were included in the universal calibration. On a return visit the following year, the beet crop had been damaged by floods and juice pol levels were far below the normal range of the factory. The NIR calibration for juice pol for that factory alone (from the previous year) did not cover the lower range of juice pol values from the flood damaged beets, and would not have been expected to return reliable pol

readings. However, the universal calibration extended across the lower range, and was used successfully to analyze juices from the flood damaged beets.

Universal calibration for sucrose in beet brei

In the past two North American beet harvests, SPRI, Inc. personnel visited beet factories to collect brei samples, record the NIR reflectance spectra and the analytical data of each individual factory. Figure 5 shows the results of NIR calibration for polarimetric sucrose in brei, and represents the pooled data from 6 factories over two years (using the wet chemical data of each factory). The calibration is based on linear regression with an $R = 0.96$ and Std. error = 0.6% w/w. Careful examination of the Figure 5 reveals that a single factory subset in the low end of the range (28 of the 242 samples) does not conform to the universal calibration. These data points are outside the range of data from the other 5 factories. The deviation in slope of this subset is believed to be due to poor quality diseased beets, and makes a profound contribution to the overall standard error. However, the range of the calibration is significantly increased by the addition of this subset. Once again the user can add to this data base to decrease the standard error, while remaining confident that the calibration will continue to produce reliable brei sucrose results (even with poor quality beets).

SUMMARY AND CONCLUSIONS

The development of universal NIR calibrations for standard sugar factory analyses appears useful because:

1. universal calibrations provide a starting point for the factory that has just acquired NIR instruments,
- and
2. addition of the factory's own data to the universal calibration will increase the accuracy of the factory's analyses in the normal operating concentration range while allowing reliable determinations outside the usual range (during unusual crop or weather conditions).

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Table 1. NIR analysis in the raw sugar refinery.

NIR ANALYSIS IN RAW SUGAR REFINERY

PROPERTY	SPECTRA	LAB	MODEL	VALID.	CALIB.
Pol in raw sugar	✓	✓	✓	✓	✓
Color in raw sugar	✓	✓	✓	✓	✓
T. Poly. in raw sugar	✓	✓	✓	✓	✓
Dextran in raw sugar (AOAC)	✓	✓	✓	✓	✓
Starch in raw sugar	✓	✓	✓		
Ash in raw sugar (conductivity)	✓	✓	✓	✓	✓
Invert in raw sugar (HPLC)	✓	✓	✓		
Moisture in raw sugar	✓	✓	✓	✓	✓
Pol in process streams	✓	✓	✓		
Brix in process streams	✓	✓	✓		
Moisture in whites	✓	✓	✓		

Table 2. NIR analysis in the cane sugar factory.

NIR ANALYSIS IN CANE SUGAR FACTORY

PROPERTY	SPECTRA	LAB	MODEL	VALID.	CALIB.
Pol in juices	✓	✓	✓	✓	✓
Brix in juices	✓	✓	✓	✓	✓
Sucrose in juices (HPLC)	✓	✓	✓	✓	✓
Ash in juices (conductivity)	✓	✓	✓	✓	✓
Invert in juices (HPLC)	✓	✓	✓		
Dextran in juices (AOAC)	✓	✓	✓	✓	✓
Fibre in cane	✓	✓	✓		
Moisture in cane	✓	✓	✓		
Sucrose in cane	✓	✓	✓		
Sucrose in bagasse	✓	✓	✓		
Fibre in bagasse	✓	✓	✓		
Moisture in bagasse	✓	✓	✓		

Table 3. NIR analysis in the beet sugar factory.

NIR ANALYSIS IN BEET SUGAR FACTORY

PROPERTY	SPECTRA	LAB	MODEL	VALID.	CALIB.
Pol in diff. & thin juices	✓	✓	✓	✓	✓
RDS diff. & thin juices	✓	✓	✓	✓	✓
Sucrose in juices (HPLC)	✓	✓	✓	✓	✓
Invert in juices (HPLC)	✓	✓	✓		
Raffinose in juices (HPLC)	✓	✓	✓		
Pol in brei	✓	✓	✓	✓	✓
Moisture in pulp	✓	✓	✓	✓	✓
α Amino N in brei	✓				
Sucrose in brei (HPLC)	✓				
Pol in thick juice	✓	✓	✓	✓	✓
RDS in thick juice	✓	✓	✓	✓	✓
Sucrose in thick J. (HPLC)	✓	✓	✓		
Pol in mol. desugariz. feed	✓	✓	✓		
RDS in mol. desugariz. feed	✓	✓	✓		

Others: brei cations; sugar color, moisture, turbidity; cossettes

Table 4. NIR analysis of diffusion (or raw) and thin juice.

Analyte	No. of samples	No. of factories	Model	Correlation coeff.	Std. error
Diffusion juice:					
Pol	211	5	LR	0.990	0.26 % w/v
Brix	211	5	MLR(2)	0.983	0.32 % w/w
Thin juice:					
Pol	228	6	LR	0.989	0.43 % w/v
Brix	228	6	MLR(2)	0.992	0.30 % w/w
Diffusion + thin juice:					
Pol	589	8	LR	0.980	0.44 % w/v
Brix	589	8	MLR(2)	0.971	0.47 % w/w

LR - linear regression at single wavelength

MLR(n) - multiple linear regression at n wavelengths

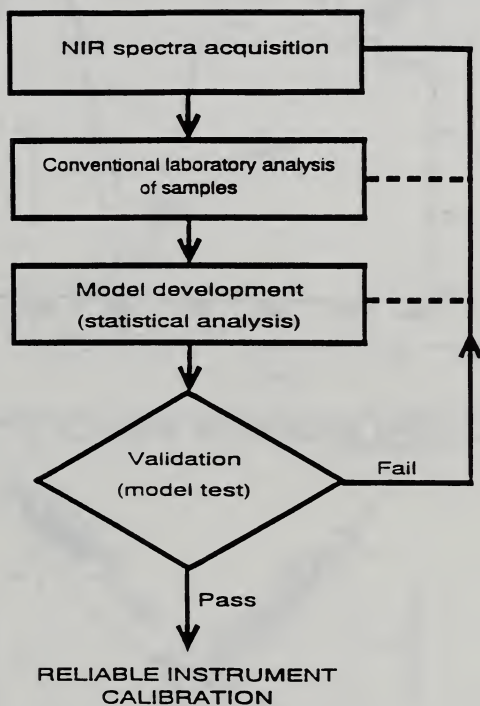


Figure 1. Scheme of NIR calibration development.

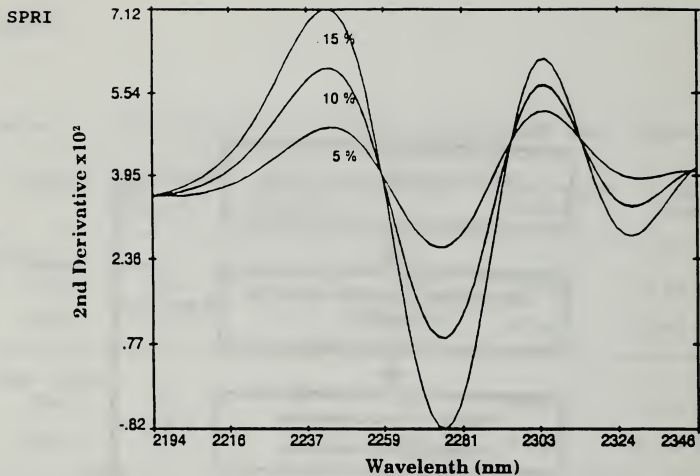


Figure 2. Second derivative NIR spectrum of pure sucrose solutions.

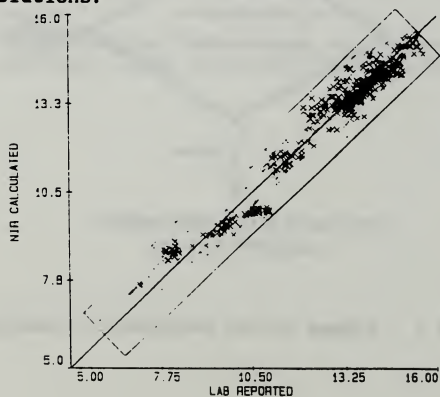


Figure 3. Universal NIR calibration for pol in beet diffusion and thin juices.

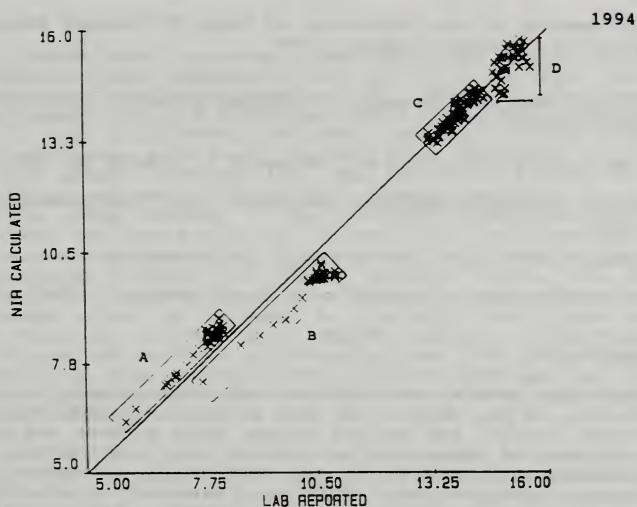


Figure 4. Universal NIR calibration for pol in beet diffusion and thin juices; fit of four factories in one year.

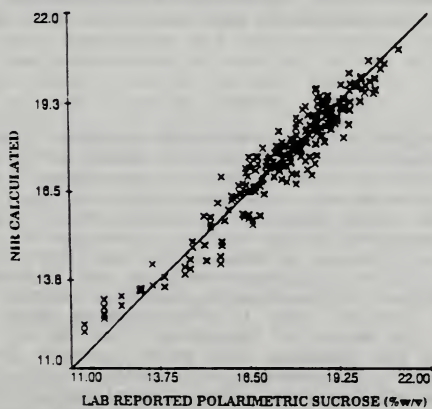


Figure 5. NIR calibration for polarimetric sucrose in brei.

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DEXTRAN ANALYSIS IN SUGAR BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Les A. Edye¹, Shaoxing Wu², Margaret A. Clarke¹ and Per J. Garegg³

¹Sugar Processing Research Institute, Inc., New Orleans, Louisiana,
USA

²Tulane University, New Orleans, Louisiana, USA

³Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden

ABSTRACT

There are several wet chemical methods available for analysis of dextran in raw sugars: the haze methods, the AOAC method (Roberts Copper method) and various enzyme based methods are among these. Results from these analyses seldom are in agreement. There are problems with specificity and sensitivity in several of the wet chemical analyses, e.g., published haze and enzyme methods do not give complete analysis for total dextran, but exhibit molecular weight and solubility selectivity.

Spectroscopic methods of analysis for dextran offer a new and more rigorous approach to an absolute determination of dextran. Spectroscopic systems and their application to dextran analysis are discussed herein.

INTRODUCTION

Dextrans are a class of extracellular microbial polysaccharides consisting of a backbone of α -D-glucopyranosyl residues with (1-6) linkages. Naturally occurring dextrans are usually branched with mostly (1-3) linkages and sometimes (1-2) or (1-4) linkages at branch points (1). The degree of branching of dextrans depends on the microbial source and varies widely among species. For example, while the dextran produced by *Betacoccus arabinosaceus* has a unit chain length of only six or seven residues and is highly branched, the dextran produced by *Leuconostoc mesenteroides* may have a unit chain length of greater than 10,000 residues with less than 5% branching (1). The degree of branching also varies among strains within species. For example, the water soluble, high molecular weight dextran (> 10,000 residue unit chain length) from *Leuconostoc mesenteroides* NRRC B-512F (ATCC 10830a) consists of 95% (1-6) linked α -D-glucopyranosyl residues with 5% α (1-3) linked D-glucosyl or isomaltosyl side chains. However, *L. mesenteroides* NRRC 523

(ATCC 14935) predominately produces a lower molecular weight, water insoluble dextran which consists of only 66% (1-6) linkages with 24% (1-3) and 10% (1-4) branch linkages (2). There is also some evidence to suggest that in a single bacterial strain the degree of branching of the polydisperse dextrans may vary across the molecular weight range, with higher probability of branching in the lower molecular weight fractions (3). The infection of sugarcane and cane milling streams with dextran forming bacteria is of concern to the sugar industry. The microbial biosynthesis of dextrans occurs via the action of extracellular dextransucrase enzymes on sucrose. Consequently, infections of sugarcane and cane milling processing streams with these bacteria cause loss of sucrose to dextran. Furthermore, high dextran concentrations in process streams effect an increase in viscosity that leads to reduced recovery of sucrose and reduced factory efficiency. Finally, dextrans and α -glucan oligosaccharides (the products of dextran hydrolysis) are dextrarotatory and their presence in raw sugar at the point of sale to refiners increases the polarimetric measurement of sucrose purity.

Currently, two methods for the analysis of dextran in raw sugar hold some favor in the sugar industry. The more commonly used haze method (4) involves enzymic removal of high molecular weight starch, ion-exchange removal of inorganic salts, precipitation of proteins with trichloroacetic acid and measurement of turbidity of a 50% aqueous ethanol solution. The haze method is not sensitive at low dextran concentrations and is specific for high molecular weight, relatively linear dextran (i.e., dextran that precipitates in 50% aqueous ethanol). The haze method was recently accepted by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) for the measurement of dextran in raw sugars. The second method is an official method of the AOAC (5), and is commonly referred to as the Roberts' copper method. The AOAC method involves quantitative precipitation of total polysaccharides in 80% aqueous ethanol, the precipitate is redissolved and selective precipitation of dextrans in alkaline copper solution is followed by colorimetric determination of sugars with the phenol-sulfuric acid reagent. While the haze method is specific for high molecular weight dextran, the AOAC method appears to be not specific to a molecular weight range. Hence, the AOAC method results are usually significantly higher than those of the haze test. In addition to the total dextran, the copper precipitate of the AOAC method may contain 1 to 4% non-dextran polysaccharides, as does the alcohol precipitate in the haze test.

Recently, Galea, et al. (6-8) have reported the development of an enzyme (dextransase)-HPLC analysis for dextran in raw sugars. In fact the development of enzymic methods for the analysis of dextrans in sugar dates back to 1974 (9), but as routine analyses these methods have held little favor with the sugar industry since

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they are technically difficult and time consuming. The current enzyme-HPLC method requires a minimum of two days per batch of samples. However, the primary intent of Galea, et al. was to establish a reference method (rather than a routine analysis) by which currently favored methods (viz., AOAC and haze methods) could be compared (8).

The enzyme-HPLC method involves quantitative precipitation of total polysaccharides in 80% aqueous ethanol, digestion of the precipitate with dextranase from *Chaetomium gracile* (10), and HPLC analysis of the isomaltose product of dextranase hydrolysis. There is little doubt that this enzyme is specific for dextran. However, the calculation of dextran concentration in the raw sugar is based on a dextran to isomaltose conversion factor. This conversion factor is determined by the action of the dextranase on Pharmacia T-series dextrans (Pharmacia Fine Chemicals) and cane dextran purified by precipitation in 50% aqueous ethanol. These dextran standards are comparatively linear with a narrow molecular weight ranges. Hence, the method fails to take into account the polydispersity and heterogeneity of naturally occurring dextrans (i.e., the method underestimates dextrans with higher branching frequencies). Consequently, it is not valid to refer to the enzyme-HPLC analysis of dextran in raw sugars as a reference method. This paper reports our attempts to develop a reference method for the analysis of dextran in raw sugars that is based on a physical measurement of dextran (using ^1H NMR) with little wet chemical preparation of the raw sugar sample.

EXPERIMENTAL

Materials: The raw sugars were from the library of sugars at SPRI and is representative of the range of dextran concentrations found in raw sugars. The stractan (Champion International Corp.) was a gift from S. Vercellotti (V-Labs, Inc., Covington, LA). All other chemicals were analytical grade.

Methods: Raw sugars (2.0 g) were each dissolved in stock aqueous solutions of stractan (0.08 gL^{-1})/dextran (0.08 , 0.16 and 0.32 gL^{-1}) mixtures so that the final volume was 50 mL. The high molecular weight fractions of an aliquot (20 mL) of these raw sugar solutions were prepared by membrane filtration (Centriprep 10 Concentrator, Amicon; 10,000 Da cut off): final volume 2 mL. For ^1H NMR spectra (500 MHz), the high molecular weight fractions were evaporated to dryness and pre-exchanged with D_2O four times.

RESULTS AND DISCUSSION

Figure 1 shows the ^1H NMR of a raw sugar (designated as sample 1 below). The dominant features of this spectrum are the proton signals of sucrose, but other signals from the minor components of raw sugar are also present. The ^1H NMR spectrum of sucrose has been fully assigned (11). For the purpose of this study we are interested in the signals from ca. 4.4 to 5.7 ppm (i.e., anomeric proton region). The region of the spectrum expanded in Figure 1 shows two doublet signals, 4.98 ppm, $J = 3.41$ Hz and 5.33 ppm, $J = 3.92$ Hz that can be assigned to the anomeric protons of dextran (based on ^1H NMR of pure dextrans and dextran spiking of raw sugars), δ 4.98 ppm is C1-H of $\alpha(1\rightarrow6)$ linked D-Glc₆ of dextran and δ 5.33 ppm is C1-H of $\alpha(1\rightarrow3)$ linked D-Glc₆ of dextran.

Direct integration of the small dextran peaks in the ^1H NMR spectrum of this raw sugars is not possible. However, after concentration of the medium and high molecular weight components ($> 10,000$ Da) by membrane filtration the dextran peaks in the ^1H NMR of this concentrate can be reliably integrated.

The quantitative dextran determination involves the use of a polysaccharide internal standard so that dextran concentration in the high molecular weight fraction can be related to dextran concentration in the raw sugar. Stractan was chosen as an internal standard since it has a molecular weight $>10,000$ Da, it is water soluble and has no ^1H NMR signals that overlap in the anomeric proton region with either dextran or sucrose. Stractan, a polysaccharide from western larch (*Larix occidentalis*), is composed of D-galactose and L-arabinose in a ratio of 6:1. The $\beta(1\rightarrow3)$ -D-Gal₆ backbone of stractan has side chains of ca. two aldose units (mostly $\beta(1\rightarrow3)$ -L-Ara, and some $\beta(1\rightarrow6)$ -D-Gal₆). The ^1H NMR spectrum of stractan shown a major signal in the anomeric proton region (4.46 ppm, d, $J = 8.3$ Hz) due to the C1-H of $\beta(1\rightarrow3)$ linked D-gal₆; other minor peaks do not overlap with the dextran anomeric proton signals.

Four raw sugar sample were dissolved in aqueous solutions of dextran and stractan; the resulting solutions contained 2000 ppm stractan (based on raw sugar), and either 1000 or 2000 or 4000 ppm added dextran (based on raw sugar). Figure 3 shows integration of the anomeric proton region of the ^1H NMR spectrum of sample 1 with 4000 ppm added dextran. The peak area ratios of the anomeric proton signals of dextran (4.89 ppm) and stractan (4.46 ppm) when plotted against the weight ratios of added dextran/stractan for the four samples are a series of parallel lines (see Figure 4). The dextran concentrations in the raw sugars can be calculated by extrapolation of these lines to the weight ratio axis. The integration results obtained from the stock solutions of dextran

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and stractan without raw sugar are shown in a fifth line in Figure 4 that intersects with the origin.

The results of the determination of dextran concentration in the four raw sugars by ^1H NMR are compared to the results of the two favored wet chemical methods in Table 1. It would appear from this preliminary comparison of methods that, at least in these four samples, the haze method underestimates dextran content in raw sugars, and that the AOAC method is in good agreement with the ^1H NMR method.

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Table 1. Comparative study of methods for determination of dextran in raw sugars.

Sample number	AOAC method (ppm)	Haze method (ppm)	¹ H NMR method (ppm)
1	1551	1058	1568
2	557	218	594
3	430	173	483
4	156	83	136

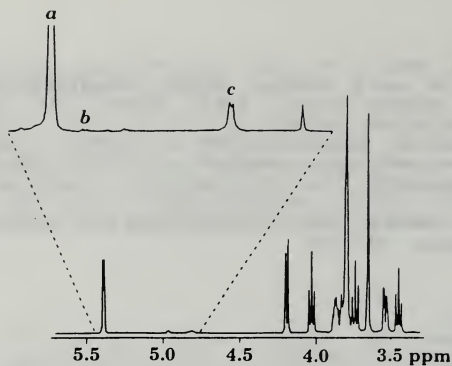


Figure 1.
 ^1H NMR of a raw sugar.

- a - 5.40 ppm, d, $J = 3.64$ Hz (sucrose, C1-H of glucose moiety)
 b - 5.33 ppm, d, $J = 3.92$ Hz (dextran, C1-H of $\alpha(1\rightarrow3)$ linked D-Glc_p)
 c - 4.98 ppm, d, $J = 3.41$ Hz (dextran, C1-H of $\alpha(1\rightarrow6)$ linked D-Glc_p)

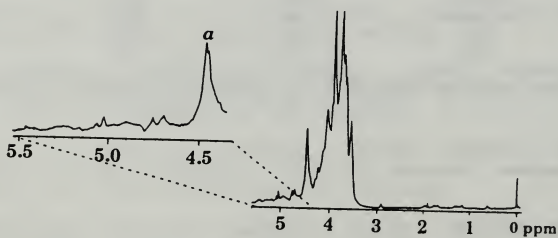


Figure 2.
 ^1H NMR of stractan.

- a - 4.46 ppm, d, $J = 8.31$ Hz (C1-H of $\beta(1\rightarrow3)$ linked D-Gal_p)

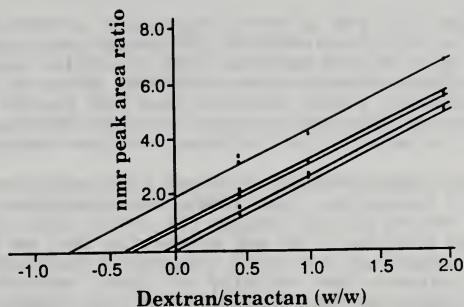


Figure 3.

Integration of anomeric proton signals in a ^1H NMR spectrum of a raw sugar containing added dextran and stractan.

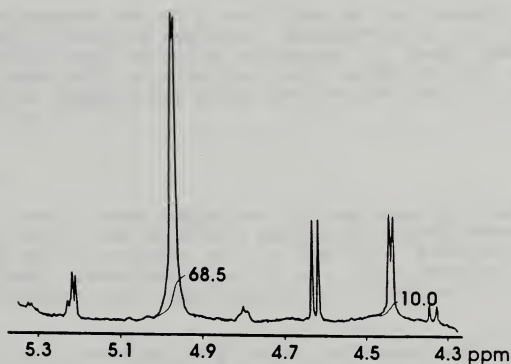


Figure 4.

Determination of dextran in raw sugars by ^1H NMR.

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DISCUSSION

Question: Thank you for a very interesting presentation on a new approach to an old problem. Do you think that two methods are needed - the haze method measures high molecular weight material, which is the part of dextran that hinders filterability, whereas the lower molecular weight dextran does not impede filtration so much. However, if you need to know the total dextran, to indicate the amount of false pol, for instance, then the new method is good.

Edye: Yes. For process control, if we understand exactly what the haze method is measuring, then there is no problem. For the sake of truth, we should try to dismiss the notion that the AOAC method over-estimates dextran. I am not suggesting that we use the proton nmr (^1H nmr) method as a routine analytical technique, but it can be used to indicate the validity of other methods, as in this presentation where we have compared the haze method and the AOAC (Roberts) method.

SENSORY PROPERTIES OF WHITE BEET SUGARS

Mary An Godshall¹, Casey C. Grimm², and Margaret A. Clarke¹

¹Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, Louisiana, USA

²USDA-ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, Louisiana, USA

ABSTRACT

Questions periodically arise about the sensory quality of white beet sugars, related to uncharacteristic and undesirable odors. Many of the volatile odor compounds identified in beet sugar arise from the nature of the sugarbeet plant and the production process. The sugarbeet is a root crop with attendant associated earthy and herbal volatile compounds. Manufacture into white sugar is accomplished via crystallization from a minimally processed syrup matrix, which may leave volatiles adhering to the sugar.

This paper discusses the odor profiling of beet sugars using gas chromatographic olfactory analysis, accompanied by the identification of several key volatile compounds by mass spectrometry. Quantitative analysis of volatile fatty acids is also discussed, with the goal of identifying steps that can be taken to improve the sensory quality.

INTRODUCTION

The need to control odor emissions in sugarbeet processing is of longstanding importance because a series of strong, characteristic odors is typically produced in the factory. Sugarbeet molasses also has strong, characteristic odors. As a result, the final white sugar product will occasionally retain traces of the process odors.

Oldfield, et al., studied pulp drier emissions and reported the presence of over 150 compounds, 82 of which were fully or partially identified (1). They found that three volatile fatty acids, acetic, propionic and butyric, accounted for the majority of the volatile material. Acetic acid concentrations ranged from 200 to 1068 mg/liter of condensate and was the largest component of the pulp drier volatiles. Other compounds identified included amines,

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aliphatic and aromatic acids, esters, pyrazines, alcohols, phenols, lactones, aldehydes, ketones and dicarbonyls.

MacLeod, et al., studied the volatile components of sugarbeet leaves while examining growth substances (2). They identified several C-6 alcohols (responsible for green, leafy odors), the growth substance phenylacetonitrile, some hydrocarbons, phenylacetaldehyde, and a few other aromatics.

Kiely and O'Driscoll measured three volatile fatty acids, acetic, propionic, and butyric, in Irish molasses and found acetic acid to range as high as 3.33% on molasses (3). Propionic acid was present at about 0.01-0.02% and butyric acid from trace quantities up to 0.98%, depending on factory location and date of production.

Tressl, et al., examined volatile substances in molasses, and identified 25 nitrogenous compounds, 14 furans and phenols, and 29 aliphatic and aromatic acids (4). Again, acetic acid was the most concentrated volatile, measuring 3800 ppm. Isovaleric acid was present at a concentration of 3.5 ppm. It is interesting to note that indole (0.05 ppm) and skatole (0.05 ppm) were also identified. These two heterocyclic nitrogenous compounds have characteristic fecal odors, but at very low concentrations, are reported to have ripe, fruity or floral odors, and are both approved for use as flavoring compounds in foods (5).

Godshall identified more than 40 volatile compounds in beet molasses, including some sulfur-containing compounds (6).

Isovaleronitrile was identified as a unique, characterizing compound from molasses alcohol, and was used to distinguish it from grain alcohol (7).

Accorsi and Blo determined volatile and non-volatile organic acids in technical sugar juices with ion exclusion HPLC, and identified two volatile fatty acids, formic and acetic acid (8). The acetic acid concentration in five process juices ranged from 5.0-17.0 ppm.

Little has been published on the volatiles of white, granulated beet sugar. Earlier work reported in 1991 by the Sugar Processing Research Institute attributed some common off-odors to volatile fatty acids of microbial origin (20).

Kaipainen, in Finland, reported several compounds responsible for odors in granulated beet sugars (9). These included p-methoxybenzaldehyde, m-methoxybenzaldehyde, m-methylanisole, n-decanal, myristaldehyde, geraniol, and citronellyl acetate. Two other compounds, 2-phenoxyethanol and BHT, were felt to be exogenous to the system, the first as an added bactericide and the latter an antioxidant found in packaging materials.

Recently, Marsili, et al., reported 26 compounds from beet sugar using two purge-and-trap techniques (10). They concluded that acetic, butyric and isovaleric acids in combination with geosmin were responsible for the odor defects noted in white beet sugars.

Table 1 lists the compounds reported in white beet sugar by the Kaipainen study (9) and Table 2 lists the compounds reported in the Marsili study (10).

MATERIALS AND METHODS

White sugars of low odor quality were obtained from European and North American sponsoring companies of Sugar Processing Research Institute, Inc. (SPRI). Sugars examined came from Europe and North America.

Three different methods were used for the examination of the volatiles in the beet sugars.

(1) An External Closed Inlet Device (ECID, manufactured by SIS, Harahan, Louisiana), interfaced to a 5880 Hewlett Packard Gas Chromatograph, was used to quantitate acetic, propionic and butyric acids. Approximately 1.5-1.6 grams of sample was introduced into the sample tube, 2 μ l water was added to the sample to facilitate distillation of the volatile fatty acids, and volatiles were purged from the sample at 130°C for 4 min. Purged volatiles were deposited directly at the head of a 30 m, fused silica DB-5 capillary column, 0.320 mm internal diameter. The separation took place at 20°C for 4 min, with a temperature ramp of 6°C per min to 200°C and held for 2 min. These conditions resulted in complete purging of the volatiles without deterioration of the sugar, which remained white.

(2) A Tekmar LSC-2000 Purge and Trap apparatus was used for olfactory detection (OD) (sniff testing) of the individual volatile peaks separated by gas chromatography. The eluent stream was split 1:2 between the detector and the olfactory port. About 10 grams of sugar was placed in the sample holder and 100 μ l water added into the loop to aid in volatilization of the odorants. Volatiles were purged from the sample at 75°C for 15 min. A 30 m DB-5 capillary column, 0.530 mm internal diameter was used. Conditions of chromatographic separation were: 35°C for 1 min; temperature ramp of 5°C per min to 250°C min; hold for 1 min.

Similar purging conditions were used with the Tekmar LSC-2000 interfaced to the GC/MS. Chromatographic conditions for MS were: 50 m DB-5 capillary column 35° C for 1 min; ramp 5°C per min to 150°C; ramp 10°C per min to 240°C; hold for 10 min to clear out the system.

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(3) Volatiles were also purged from the sugar using a Short Path Thermal Desorption apparatus (SPTD, Model TD-2, manufactured by Scientific Instrumentation Services, Inc., Ringoes, New Jersey) interfaced to a 5988A Hewlett Packard GC/MS system. About 0.8-1.0 g of sample was placed in the sample tube with 2 μ l water added and purged for 10 min at 110°C. Conditions of chromatographic separation were: 0° C for 10 min; temperature ramp of 5°C per min to 100°C, followed by a temperature ramp of 10°C per min to 250°C for 15 min to clear out the system.

RESULTS AND DISCUSSION

When sugars were received for odor evaluation, the over-all odor was noted qualitatively. Table 3 lists some of the types of odor impacts that have been noted for representative sugars of low quality with recognized odor problems.

The three purging techniques described in the methods section were used to obtain volatiles for gas chromatography, each with its own advantages and limitations. The Tekmar LSC-2000 has the advantage of accommodating a larger sample size, 10 or more grams, compared to either the ECID or the SPTD, which can accommodate a maximum of about one gram of sample. The Tekmar is prone to carry-over and contamination from previous samples or standards because of its valving and the lengths of tubing involved in sample transfer.

The small sample size is perhaps the greatest limitation of both the ECID and SPTD. This can be overcome by purging volatiles from a larger sample into some type of adsorbent trap and then desorbing the trapped volatiles from the adsorbent material by either ECID or SPTD. This, however, results in a more complicated and time-consuming method.

It is also evident that each technique favors the extraction of certain volatiles and fails to extract others. The Tekmar is better for revealing the earlier-eluting volatiles, while they tend to be lost with the SPTD. The ECID seems to be especially well suited for revealing the volatile fatty acids. With the addition of a small amount of water to aid in steam desorption, the ECID extracts VFA's more consistently than the two other methods. Propionic and butyric acids were difficult to detect except with the ECID or by preconcentrating the volatiles onto an adsorbent trap.

A wide variety of odors was noted on GC odor-profiling of white sugars. Figure 1 shows the GC profile of the volatiles from a sugar using the Tekmar. The odors noted for the eluted peaks are noted on the chromatogram. The goal, of course, is to be able to

identify the peaks, especially those with the highest olfactory impact. It can be noted that sometimes a very powerful odor is accompanied by a very small peak, showing that the human nose can be a more sensitive detector for some compounds than the FID of the gas chromatograph. In other instances, a moderate to large GC peak will produce little or no discernible odor to the nose.

Figure 2 shows an ECID profile of the volatiles from approximately 1.5 g sugar. Figure 3 shows an ECID chromatogram of white sugar in which the volatiles from 11 g of white sugar were purged onto a trap containing Tenax-GC. The trap was then thermally desorbed using the ECID. This chromatogram shows the enhanced recovery of volatile fatty acids. Figure 4 shows a portion of the chromatogram (from 30-40 min) of the volatiles in approximately 1 gram of white sugar using the SPTD. Figure 5 shows the volatiles from 16 grams of white sugar trapped on Tenax-GC and desorbed using the SPTD.

Table 4 shows the results of volatile fatty acid quantitation using the ECID. Acetic acid concentration ranged from 0.4 to 5.0 ppm; propionic acid concentration was 0 to 2.2 ppm; and butyric acid concentration was 0 to 0.3 ppm. Marsili, et al., (10) found butyric acid levels of 43.4 to 990 ppb (0.04-0.999 ppm).

Table 5 shows the compounds that have been identified in several representative sugars in this study. Butyric acid, isovaleric acid (3-methyl-butanoic acid) and nonanal, in combination, are thought to be the main contributors to the off-odors noted in these sugars. Combinations of these compounds would account for the cheesy, dairy, and floral/green odors that characterize many of the sugars studied. Isovaleric and butyric acids have similar odors, but isovaleric is more powerfully cheesy, and butyric acid in low concentrations can be reminiscent of dairy products such as milk or butter. Marsili found levels of 0.02 to 0.08 ppm isovaleric acid in the sugars he studied (10).

While acetic acid is the major volatile constituent, it does not contribute significantly to the odor profile because of its rather high detection limit of 54 ppm (15). It could, however, be used as a marker compound for quality determination.

Geosmin and Other Earthy Odors in Sugars. Geosmin (1,10-trans-dimethyl-trans-(9)-decalol), shown in Figure 6 has been reported to be a major volatile constituent of red beet (16,17). Marsili reported it was present in the sugars he studied, although it was present below GC, MS and olfactory detection levels (10). Geosmin has a powerful, earthy odor and is produced in both earth and water as a result of the action of Actinomycetes species and blue-green algae (18). In this study, we were unable to find geosmin or methylisoborneol (MIB), another common earthy odorant. Taste panelists at the Southern Regional Research Center, who are

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specifically trained to note and distinguish the odors of geosmin and MIB, could not identify either geosmin or MIB odors in the sugars examined. Geosmin and MIB could not be perceived by OD nor were they identified by MS in a Tenax-concentrated sample (Figure 5), even with selected ion monitoring using masses 112 and 182, specific for geosmin, and masses 95 and 168, specific for MIB. Given that the odor threshold of geosmin is only 0.025 to 0.1 ppb, it is not surprising that it is difficult to detect.

However, several mushroomy, earthy odors were clearly noted on OD of the sugars, as well as an odor reminiscent of Brie cheese. Mushroomy, earthy odors are contributed by a number of C8 alcohols, ketones, and aldehydes (11,12). C8 compounds are produced by the action of lipoxygenase enzymes on fatty acid precursors (13). Lipoxygenase enzymes are ubiquitous, and the resulting volatiles could be from the action of beet plant enzymes or microbial enzymes on the beet fatty acids. MS showed indications of C8 compounds, but in concentrations too low to allow identification of any compounds with confidence.

Trials on mixing various combinations of volatiles into an odorless white cane sugar, approached, but did not duplicate the odor of the sugars. These included mixtures of solutions containing geosmin, nonanal, 2-undecenal, isovaleric acid and C2-C5 acids at 1 ppm or less.

Sources of the Problem and Possible Solutions. There are multiple causes for the development of odors in beet processing:

The soil in which the beet root resides can contribute earthy and musty compounds from the action of soil microorganisms, such as *Penicillium*, algae, and other fungi and bacteria. Adhering soil will result in transportation of these compounds into the process. These odorants would be more prominent during times when there is high rainfall and the soil is wet for a prolonged period of time.

The sugarbeet juice itself is the source of reactive precursors, such as amino acids, other nitrogenous compounds, and sugars which can react or degrade during processing to form short-chain aldehydes, pyrazines and furans. The resulting odors tend to be slight and are sweet, caramel-like or nutty. An example compound is furfural.

Lipoxygenase enzymes in the beet root and/or microorganisms can oxidize fatty acids to corresponding long-chain aldehydes (especially C8 to C10) and alcohols with powerful odors. These lipid oxidation products can produce green, as well as mushroom odors (19).

Microbial infection during storage of the beets or during processing will produce volatile fatty acids (20). A bacterial infection somewhere in the process, such as in the wash water or any other low-concentration process stream, could result in the production of both volatile fatty acids and polysaccharides. A previous study had shown a correlation between high odor and high total polysaccharide concentration in commercial, granulated sugar (14). In this study, the odorous sugars were found to have polysaccharide levels >100 ppm and pH around 6.5. All of the high-odor sugars also had measurable concentrations of acetic acid, which may be used as a marker compound for a quality problem.

The bulk of the volatile compounds present in sugar are concentrated on the surface of the crystal, much of it in the syrup layer. Even under the best of conditions (ie, no infection in the factory, clean beets in good condition), insufficient washing of crystals may result in failure to remove plant and process-derived odors.

We have found that washing the crystals with solvents such as methylene chloride or methanol removed all odors from the white sugars. This course of action cannot be recommended, but we have also found that a warm, dry stream of helium strips all the volatiles from the crystal surface. It is therefore possible that purging of odorous sugars with dry air, as in conditioning, could help lessen the odor load, as could repeated transfer of sugar (20).

Preventive measures were detailed in an earlier presentation (20). Preventive or corrective measures may include:

1. Avoid excessive green tops coming into the process. This will contribute not only plant-derived odors but colorants and turbidity, as well. If the beets are stored or piled for several days, the green tops will ferment and contribute silage odors along with some of the more unpleasant "barnyard" (protein decomposition) odors.
2. Beets should be adequately washed of earth.
3. Check for areas of microbial contamination in the factory and the beets and take corrective steps. Microbial contamination causes not only undesirable odors but also sugar loss and high polysaccharide concentrations in the resulting white sugar.
4. Increase washing time on white sugars.
5. For emergency control, add powdered carbon in the final filtration step.

CONCLUSIONS AND SUMMARY

In conclusion, and to summarize, this study has identified several compounds that contribute to the odor of beet sugars. Chromatography and sniff testing by olfactory detection show that many more compounds remain to be identified. Several categories of odors impact the sensory quality of sugar:

1. The volatile fatty acids are the most important, both quantitatively and qualitatively. These include acetic, propionic, butyric and isovaleric acids. The latter two are the most important from an organoleptic viewpoint. Their origin may include bacterial infection of beets in storage or infection in the process, and is usually accompanied by elevated polysaccharide levels (>100 ppm in white sugar) and lower pH. Prevention requires rigorous sanitation, and may require extra centrifugal washing.

2. Silage, straw, earthy-beety and mushroom-like odors are the next most important to the over-all odor impact. On OD chromatography, these often give small peaks or no peaks but are distinct to the human nose. Sources may include fungal metabolites from soil organisms. Geosmin may or may not be in this group of compounds. (This study did not find geosmin, but it has been reported previously by other workers.) Beets brought into storage and processing should be as clean as possible.

3. Green, floral, citrus and other plant-like or herbaceous and aromatic odors are contributed by a series of alcohols and aldehydes, the most important of which is probably nonanal. Possible sources include the activity of lipoxigenase enzymes on fatty acids as well as deterioration of leaf material. Avoidance of leafy tops is recommended. Aeration of granulated sugar may help remove these from the surface of the crystal.

4. Peanut, chocolate, roasted nut and meaty odors represent reactions of nitrogen and sulfur-containing amino acids and other nitrogenous compounds with reducing sugars. They could be, for example, pyrazines and sulfur heterocycles. These provide the faint musty, nutty background of some sugars, but, in general, are not very important to the over-all odor profile.

5. Sweet, caramel-type odors, contributed by furfural, diacetyl and possibly other furan compounds are responsible for a faint, sweet, background odor note. These represent sugar breakdown products and are not very important to the odor profile. Their production is minimized by good manufacturing practices that avoid sugar breakdown.

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Table 1. Volatile compounds identified in white beet sugar by Kalpainen (9)

p-methoxybenzaldehyde	
m-methoxybenzaldehyde	
m-methylanisole	
n-decanal	
myristaldehyde	
geraniol	
citronellyl acetate	
2-phenoxyethanol	(source: biocide)
BHT	(source: antioxidant in packaging)

Table 2. Volatile compounds identified in white beet sugar by Marsili, et al. (10)

propanoic acid	carvone
butyric acid	pentane
isovaleric acid	furfural
2-butanol	furfuryl alcohol
3-methyl-2-butanol	chloroform
2-pentanol	benzaldehyde
2-ethylhexan-1-ol	butyl acetate
dichlorobenzene	acetic acid
1-octene-3-ol	3-methylbutanal
2-methylhexylpropanoate	2-pyrrolidinone
5-methyl-2(3H)furanone	geosmin
2,5-dimethylpyrazine	2-octanol
tetrahydro-2,3-dimethyl-2-furanol	
2-methyl-2-ethyl-3-hydroxyhexylpropanoate	

Table 3. Odor characterization of some representative white beet sugars with quality problems due to off-odors.

Sugar	Types of odors noted in granulated sugar
A	Silage, geranium leaves, butyric acid
B	Over-ripe fruit, silage, butyric acid
C	Floral, butyric acid
D	Barnyard
E	Red beets, silage, straw
F	Bacon or cured meat
G	Silage, barnyard, slight fecal
H	Milky, cow-like, straw

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Table 4. Volatile fatty acids in white beet sugar from one manufacturer over several campaigns .

1990 Campaign			
Sugar	ppm Acetic	ppm Propionic	ppm Butyric
1	5.0	1.2	0.05
2	2.5	1.3	0.2
3	2.5	0.6	0.1
4	0.6	1.1	0.04
5	0.8	0.9	<0.01
6	0.5	N.D.	N.D.
7	3.1	1.6	0.3
8	3.7	N.D.	0.2
9	3.7	N.D.	0.1

1991 Campaign			
Sugar	ppm Acetic	ppm Propionic	ppm Butyric
1	2.3	0.8	<0.01
2	0.6	2.0	<0.01
3	1.3	1.4	0.04
4	3.0	2.0	0.1
5	2.6	1.6	0.05
6	2.2	2.0	0.05
7	1.8	0.6	<0.01
8	1.5	0.1	N.D.
9	1.6	1.7	<0.01
10	1.3	0.7	<0.01
11	1.4	0.3	0.02
12	1.6	2.2	0.05

1992 Campaign			
Sugar	ppm Acetic	ppm Propionic	ppm Butyric
1	0.5	0.7	N.D.
2	0.6	1.1	0.02
3	0.5	0.7	<0.01
4	0.4	0.3	N.D.
5	1.2	0.4	0.05
6	1.4	0.6	0.03
7	1.5	0.9	0.05
8	1.6	0.6	0.03
9	1.5	0.1	0.01
10	1.4	0.8	0.09
11	1.5	2.2	0.02
12	0.9	0.8	0.04

N.D. = Not detected

Table 5. Compounds identified in the volatile profile of representative white beet sugars

<u>Compound</u>	<u>Type of odor*</u>	<u>Method of ID</u>
Diacetyl	Butterscotch	RT, OD
3-Methyl butanal	Chocolate	RT, OD, MS
Acetic acid	Acidic	RT, OD, MS
Propionic acid	Acidic	RT, MS
Butyric acid	Rancid, butter	RT, OD, MS
Isovaleric acid	Rancid cheese	RT, OD, MS
Octanal	Green, sharp, citrus	RT, OD, MS
Nonanal	Citrus, green	RT, OD, MS
Decanal	Floral, citrus, geranium	RT, MS
2-Undecenal	Rose, citrus, sweet	RT, MS
Furfural	Caramel	RT, OD, MS
Benzaldehyde	Almonds, sweet	RT, MS
Acetophenone	Floral, almonds	RT, MS
5-HMF	No odor; sharp taste	MS
2-Decene-1-ol	---	MS, RT

Compounds Tentatively Identified

C3 or C4 acid ester	Fruity	MS (Tentative)
2-Octen-1-ol	Mushroomy, earthy	MS (Tentative)
2,3-Butanediol	---	MS (Tentative)
7-Methyl-5-octen-4-one	---	MS (Tentative)
Hydrocarbon (>20C)	---	MS (Tentative)

* Odors of some compounds are strongly dependent on concentration. For example, nonanal out of the bottle has a fatty, nondescript odor, but as it evaporates on a surface, such as paper, it becomes strongly citrus-like. In very low concentrations, as when sniffed at the sniffer port of the GC, it has a musty-green, penetrating odor.

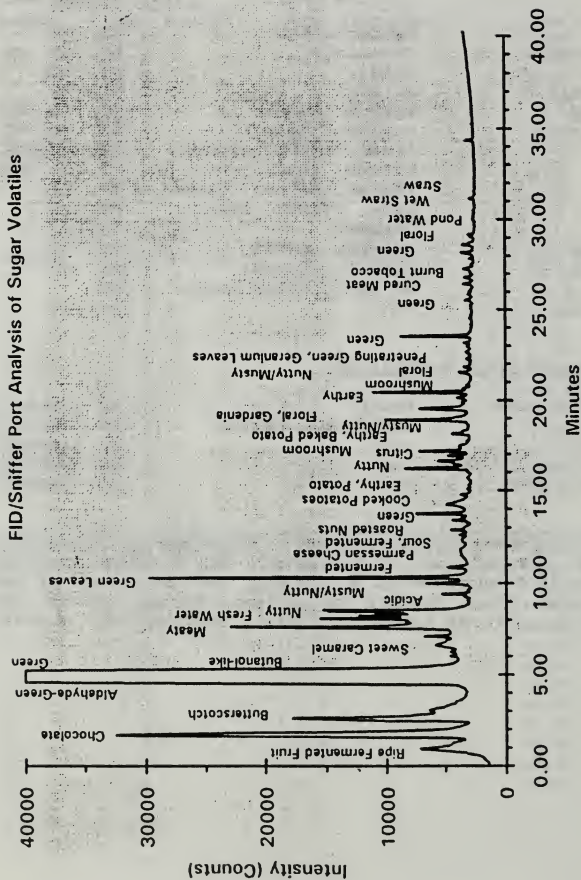


Figure 1

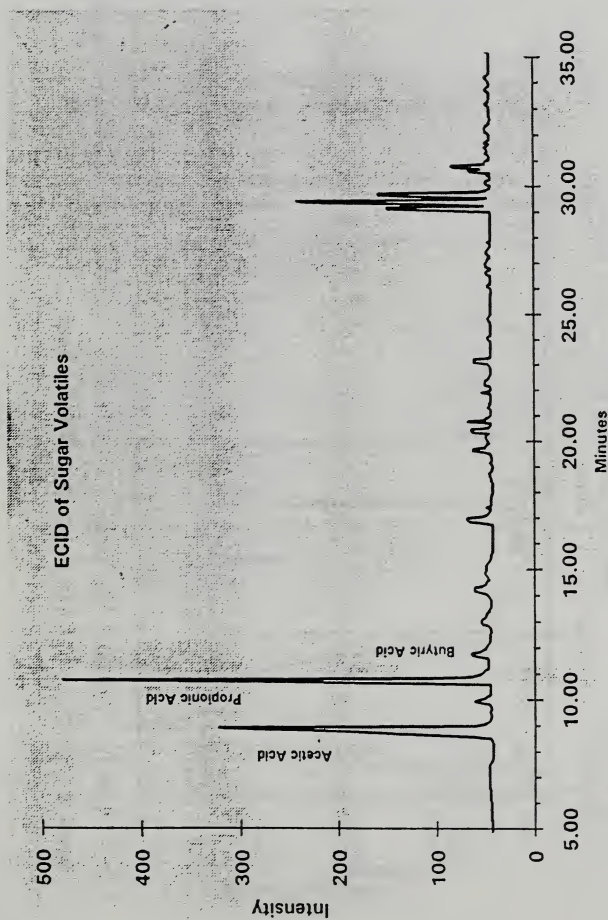


Figure 2

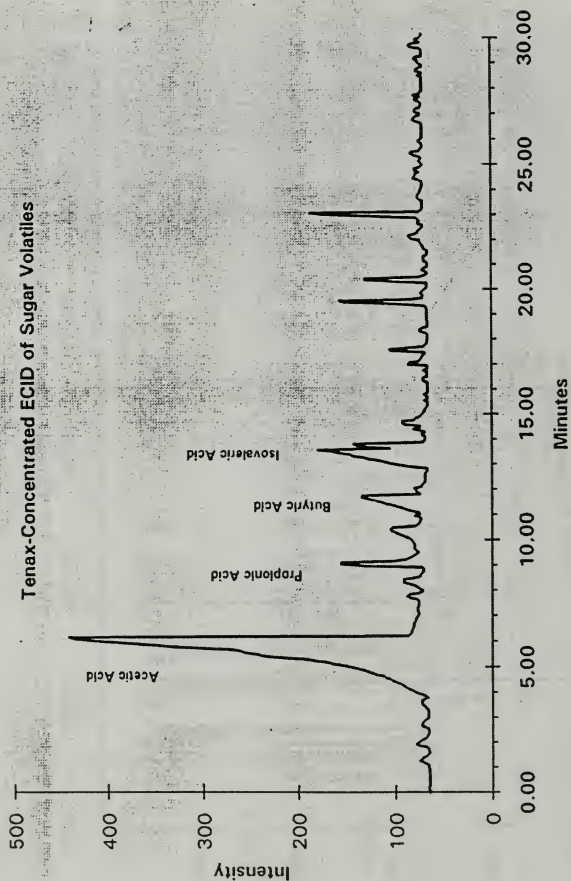
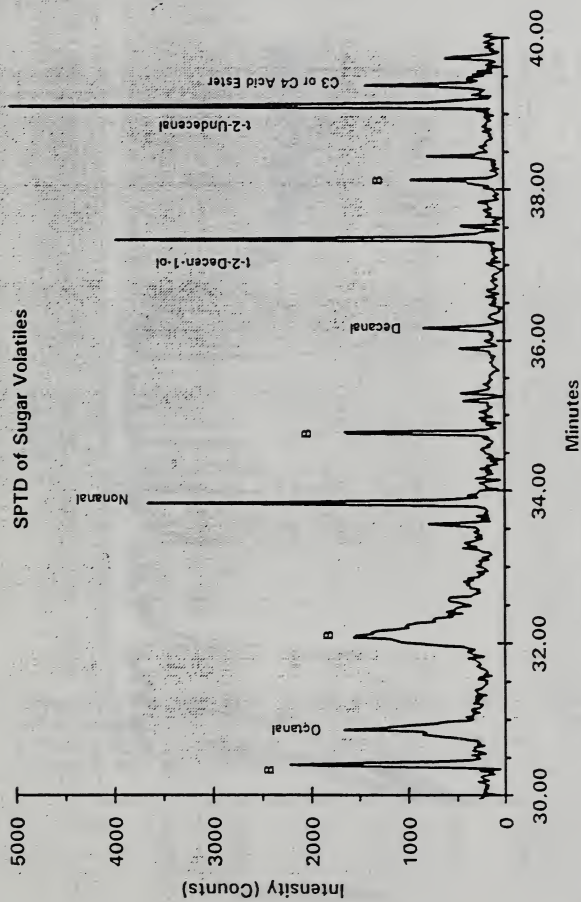


Figure 3



B = Blank/Column Peaks

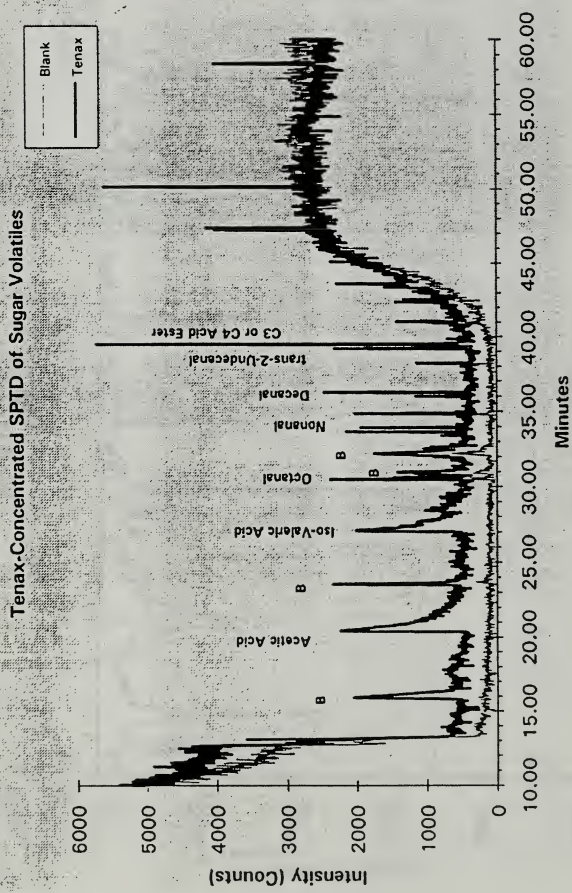


Figure 5

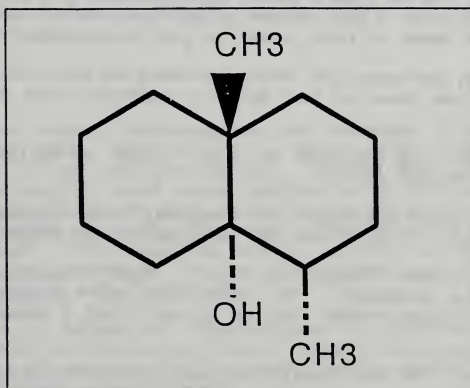


Figure 6. Structure of geosmin. (Geos = earth; osme = odor)
Threshold odor concentration = 25 - 100 parts per
trillion. Decomposes in acid to odorless compounds.
Geosmin was not identified in this study.

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DISCUSSION

Question: Thank you for your nice talk. Have you tried any technique to use a larger sample of sugar - the largest you mentioned was about 15-16 g of white sugar.

Godshall: Yes, we'll have to find a way to do that. One of our current goals was to find a rapid analysis for the fatty acids, but now we need to find a way to use larger samples to look at the tiny peaks. We'll have to find a way to pre-concentrate on polymers.

Question: Yes, because now there are ways to use 100 or even 500 g samples, but you have to use dynamic headspace and concentrate on Tenax.

Godshall: Yes, and perhaps on other types of polymers, such as graphitized carbon.

Question: Have you collected air samples, from indoors and outdoors of the factory, to find out the sources of the odors?

Godshall: We haven't done that yet. That's certainly something that could be planned. We have some air samples trapped on graphitized carbon tubes, but we haven't had a chance to look at them yet.

Question: Yes, the difficulty is that you usually have to make a solvent extraction - it's difficult because solvent is not good - you lose the volatile compounds.

Godshall: What solvent do you use?

Question: I'm trying to concentrate them on Tenax. Solvent is not good because you lose the volatile compounds. I'm using thermal desorption.

Have you studied any molasses samples - the raw materials.

Godshall: There is a reference in which we looked at molasses samples.

Comment: In our experience in British Sugar, you should study carefully the quality of all the water that comes into contact with the sugarbeet, including the transport water.

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USE OF DEAE-BAGASSE TO REMOVE COLOR, TURBIDITY AND POLYSACCHARIDES IN SUGAR MANUFACTURE

Earl J. Roberts, Margaret A. Clarke and Les A. Edey

Sugar Processing Research Institute, Inc., New Orleans, Louisiana
USA

ABSTRACT

On the laboratory scale, DEAE-bagasse was observed to remove color, and "turbidity" (Ref.: Roberts et al, Proc. SPRI, 1992, pp. 232-241, from cane juices and raw sugars. Nature and composition of color and "turbidity" have been studied with the use of DEAE-bagasse. Suspended solids were isolated and analyzed. The effect of suspended solids on color formation after heating and evaporation was studied. DEAE-bagasse showed ability to remove "color precursors." The nature of "color precursors" isolated by DEAE-bagasse is reported and discussed.

On pilot scale, a column of DEAE-bagasse was put on stream in a sugarcane factory on clarified juice. Results on removal of color and turbidity, and change in flow rate and pressure drop, after column regeneration, are presented.

INTRODUCTION

In 1992, the preparation of a complex of sugarcane bagasse with diethylaminoethyl (DEAE) chloride, and its use for removal of color and other components from sugars, syrups and liquors, was reported (1). Subsequently, preparation of this, and complexes of other agricultural residues with DEAE, have been submitted as a patent application. Since the 1992 paper, studies on DEAE-bagasse treatment of cane juice have continued, both in the laboratory for determination of non-sugars removed by DEAE-bagasse, and in the raw sugar factory, on pilot scale treatment of clarified sugarcane juice. Results of these studies (which are not complete and will be continued in the 1994-95 harvest season) are reported here.

In our search for a substitute for lead acetate for laboratory clarification of sugarcane juices, and other products, for polarization measurement (4), it was found that diethylaminoethyl cellulose was very effective in removing color and turbidity from raw sugar solutions. It was also effective in removing color and turbidity from thawed cane juice which had been frozen but was not effective in removing color and turbidity from unfrozen juice.

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Because the DEAE-bagasse compound (1) has been shown to remove color from cane products, it was of interest to test DEAE-bagasse as a clarification agent for sugar solutions.

Literature reports (2) that removal of turbidity from frozen syrup from clarified cane juice resulted in 40% reduction in color in the sugar made from the syrup. The effect of freezing on the syrup is not stated; effects of freezing on juice and syrup turbidity will be discussed later in this paper. A well known U.S. refiner has observed (4) that bagacillo content of raw sugar is directly related to color formation in that sugar in the refining process. Because of these reports, and our own observations (1), it was of interest to examine the composition of cane juice turbidity, or suspended solids. Some analyses of suspended solid material has been reported in the literature (2,5) but the nature of carbohydrate material associated with this has not been previously determined. Organic non-sugars in the suspended solids fraction, other than lipids (2,5) have not been determined.

EXPERIMENTAL - METHODS AND MATERIALS

DEAE-BAGASSE (DEAEB) treatment of raw cane juice

1000 ml of thawed raw cane juice (Brix 13.0), from hand-cleaned cane, was filtered through a coarse paper of 25 μ porosity. It was then passed through a 50g column of DEAEB. The effluent had little color and no observable turbidity. It was evaporated to 70 Brix on the rotary evaporator and was set aside to crystallize at room conditions. Crystals were filtered on a 100 mesh screen. Sugar and run-off syrup color at pH 7, are: sugar, 929 ICU; syrup, 5078 ICU.

In another experiment, using clarified factory cane juice, sugars made from juices treated with DEAEB were compared for color. Results showed that sugar from DEAE-treated juice had a color of 4295 (run-off syrup color of 17,480), while sugar boiled from the same juice without treatment had a color of 8988 (run-off syrup 114,256 ICU).

Separation of turbidity material from raw cane juice

An amount of 1000 ml of raw cane juice (13 Brix) was filtered through 25 μ porosity paper. The juice was centrifuged at 40,000 g for 20 min. Sediment was suspended in water and dialyzed for 100 h at 12,000 mw cutoff. The material in the bag was concentrated, yielding 1g of solid material (0.1% on juice, or 0.8% on solids).

Extraction of lipids from turbidity

An amount of 0.5g of juice turbidity was extracted with five 20 ml portions of chloroform in a weighed funnel. The insoluble material was dried and weighed. The extracted material amounted to 20% of the total weight.

Elemental analysis of turbidity from raw cane juice

The turbidity material from raw juice was subjected to elemental analysis and the results are shown in Table 1.

Hydrolysis of turbidity material from raw cane juice

An amount of 50 mg of turbidity material was hydrolyzed with trifluoroacetic acid at 120°C for 1 h. The acid was removed and the residue was analyzed for sugars by GC, to analyze the carbohydrate portion (18%) of the turbidity, as shown in Table 2.

Turbidity of factory clarified juice

An amount of 2500 ml of factory clarified juice (15 Brix), thawed, was centrifuged at 40,000g for 20 min. Sediments was suspended in water and dialyzed for 100 h at 12,000 mw cutoff. The material in the bag was concentrated and freeze dried, yielding 1.2g of solid material (0.05% on juice; 0.3% on solids). Carbohydrate composition determined by trifluoroacetic acid hydrolysis and GC analysis, is shown in Table 2.

Effect of chloroform extracted material on color formation

The material extracted from 0.5g of turbidity (0.1g) was added to 500 ml of 13 Brix refined sugar solution. The solution was evaporated to 70 Brix in the rotary evaporator and was set aside to crystallize. Crystals were filtered off on a 100 mesh screen and color was determined.

Original white sugar	16 ICU
Run-off syrup	814 ICU
Crystalline sugar	205 ICU

Addition of other extracts from turbidity material (lipid-free turbidity; "non-hydrolyzable" turbidity, i.e., lipid, protein and carbohydrate free) did not show significant increase in color in sugar.

Factory Trials with DEAE bagasse

An insulated metal column 5 in in diameter and 5 ft long, was loaded with five (5) lb (dry) of DEAE-bagasse, of size retained on

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a 30 mesh screen. The column dimensions were chosen to scale with a full size column, already available. The column was mounted beside the clarified juice receiving tank. Heating the column was not found to be necessary; insulation was sufficient.

Clarified cane juice at 95-98°C was passed through the column at the rate of 5-6 liter per minute under 1-2 psi pressure for 2 h. The column was then allowed to run unattended for 1 h; at this time, pressure fluctuated (from 20-30 psi), and flow rate dropped to 4-5 liters/minute. In repeat trials, the initial flow rate and pressure drop were maintained for 2-3 h, and then pressure increased. After 4 h (or 6 h, on repeats), the column was regenerated with 5% sodium chloride solution and was washed with several bed volumes of water.

After regeneration, in each trial, pressure drops were high (25-30 psi) and flow rates were unacceptably low (<1 liter/min) although treated juice was low in color and turbidity. Trials with back-flow washing and back-flow air under pressure did not solve the problem.

Results from several trials are shown in Table 3. Samples were taken every 20 minutes.

RESULTS AND DISCUSSION

Removal of color and turbidity from cane juice: Tests showed that DEAEB removed color and turbidity from fresh cane juice but more effectively from frozen cane juice. It is proposed that freezing of cane (or sugarbeet) juice causes neutralization of suspended solids: as the juice is cooled down, calcium salts that are more soluble in cold than in hot solution e.g. calcium sulfate, go into solution. The newly dissolved cations and anions neutralize charged suspended particles, converting these from suspended solids to precipitable solids. When the frozen sample is thawed, these solid particles may then be filtered off; they no longer participate as colloids, though they may still contribute to the suspended solids or turbidity fraction. It has long been observed that frozen juices (both sugarcane and sugarbeet) are much easier to clarify and to filter than unfrozen or fresh juices. The above theory, based on increasing solubility of some calcium salts with decreasing temperature, may provide an explanation.

Syrup turbidities, or suspended solids, have similarly been observed to be greater than the combined turbidities of their raw sugar and run-off syrups. A similar explanation is proposed, based on the more normal solution behavior of salts that are increasingly soluble with increasing temperature. As the syrup is heated in the vacuum pan, slightly soluble salts dissolve and form ionic bonds

with charged components of suspended solids or turbidity. These former turbidity components lose their colloidal charged, nature and can reappear as precipitable solids in raw sugar (solution) or run-off syrups.

In experiments on removal of turbidity from juice or sugars, and attempts to resuspend the material in white sugar solutions, difficulties in re-suspension were encountered. It was thus not possible to recreate turbidity removed from juice or raw sugar as a suspension in a solution of white sugar to study the effect of the turbidity on color formation. It is thought that the neutralization reactions described above remove charge from the formerly suspended particles and thus prevent re-suspension. Trials with ultrasonic treatment improved solubility but not suspension of the turbidity. Tests on DEAE-bagasse clarification of fresh sugarcane juice samples for polarization will continue in the 1994-95 crop year.

Composition of turbidity isolated from sugarcane juice: Juice suspended solids showed an average of 20% lipid material in raw cane juice and 12-13% in clarified juice. Lipid material, including fatty acids and glycerides from sugarcane wax, and phospholipids from cell walls, would be expected to be charged and contribute to turbidity. This fraction, the chloroform extract fraction, forms significant color on heating, and contributes to sugar color. This observation is relevant to comparisons of sugar quality from burned and unburned cane. About half the lipids materials is removed in juice clarification. It is to be expected that syrup clarification will remove more lipid material (as well as many other non-sugars) and therefore should be a desirable addition to processing of unburned cane. Further crystallization experiments on turbidity factors are in process.

Elemental analysis of the turbidity from raw sugar and clarified juice shows removal of approximately 50% lipid, protein and iron in clarification, but little change in total ash or silicate components. The carbohydrate fraction of suspended solids before and after clarification shows the presence of indigenous sugarcane polysaccharide (ISP), bagacillo (shown by xylose) and glucans (from starch and dextran), in similar proportions. Standard factory clarification, these results confirm, does not remove much polysaccharide material.

Factory pilot trials: results look promising for performance of DEAE on clarified juice: Fresh DEAE in a long, narrow column (not ideally proportioned), treated 600-800 bed volumes of juice before regeneration. However, trials after regeneration (with 5% brine) were not successful because pressure drop quickly became too high. Particle size (retained on 30 mesh) is apparently too fine, and packs down in the high ionic strength regenerant. Mud and fines

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collected on top of the column, but did not appear to have a large effect on the pressure drop. Breakdown of particles in the column may create fines that block the bottom of the column. Breakdown may result from normal attrition or from separation of cellulose from the particles.

Trials with larger particle size material and different filtration systems are planned but, because frozen juice does not behave in the same way as fresh juice, must wait for the next cane crop.

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Table 1. Elemental analysis of the turbidity from raw and clarified cane juice.

	% in raw cane juice	% in clarified juice
N	4.05 (25% as protein)	2.38 (14.8% as protein)
P	0.71	0.57
Ca	0.51	1.78
Mg	0.21	0.20
Al	0.67	1.20
Fe	2.7	1.04
Si	3.59	8.12
S	0.30	0.20
Sulfated ash	15.38	29.22
Carbohydrate	18.00	21.7
Lipids	20.0	12.6
Total	66.12	87.2

Table 2. Composition of carbohydrate portion of turbidity (normalized composition).

Sugar	% in raw juice turbidity carbohydrates	% in clarified turbidity carbohydrates
Arabinose	11.5	2.6
Rhamnose	0.9	0.2
Xylose	5.5	1.0
Aconitic acid	0.5	0.1
Galactose	5.5	1.2
Glucose	55.8	12.1
Uronic acid	3.2	0.7
Unknown	17.1	3.7

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Table 3. Factory trials: color and turbidity removed.

Sample	flow, l/min	psi	Color (ICU)	Turbidity (ICU)	% removed color turbidity	
Trial 1						
1 on column	5-6	1-2	10260	1648		
off column			6025	540	41	67
5 on off	5-6	1-2	10512 9704	1356 734	12	46
6 on off	5-6	1-2	11390 10455	2046 726	37	65
7 on off	5-6	1-2	11257 9560	2537 1414	15	44
Trial 2.						
0 on column off	5-6	1-2	8868 1387	2149 184	84	92
1 on off	5-6	1-2	9313 3966	2013 551	57	73
3 on off	5-6	1-2	8437 5821	2176 1086	31	50
6 on off	5-6	1-2	8896 7513	2586 316	16	88

Table 4. Factory trials: removal of color and turbidity after regeneration.

Time	Flow, l/min	psi	ICU color	ICU turb	% removed color	Turb
0 on column off column	0.5-0.8	25-30	8868 1387	2149 174	84	92
1 on off	"	"	9313 3966	2018 551	57	72
4 on off	"	"	8737 5562	2130 1533	36	28
6 on off	"	"	8896 7513	2586 1325	15	48

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LEUCOANTHOCYANINS, ACONITIC ACID AND TURBIDITY IN SUGARCANE - AN UPDATE ON CANE COLORANTS

Mary An Godshall¹ and Casey C. Grimm²

¹Sugar Processing Research Institute, Inc., 1100 Robert E. Lee
Blvd., New Orleans, Louisiana, USA

²USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee
Blvd., New Orleans, Louisiana, USA

ABSTRACT

This paper summarizes recent findings on the presence of leucoanthocyanin and aconitic acid bound to the high molecular weight colorant-polysaccharide complex in sugarcane juice and in raw cane sugar. Also discussed are recent findings on a very high molecular weight component (called VHMW Peak 1) in cane juice that contributes some turbidity in raw sugar. These three components show concentration differences in cane varieties and also some sensitivity to growth regulators. All three carry over to the raw sugar and contribute significantly to the color.

INTRODUCTION

Raw sugar color can be roughly divided into two broad categories: color that enters the factory through the cane juice from the sugarcane plant and color that is formed in process through degradation reactions (1,2). This scheme oversimplifies the issue since some colorants, such as Maillard and melanoidin colorants result from a combination of the reaction of cane juice colorant precursors and processing conditions. Most colorants deriving from the sugarcane plant are not colored in the original plant state, but become colored through enzyme catalyzed (e.g. phenol-oxidase) reactions occurring in extracted sugarcane juice.

While little work has been done to quantitate the relative contribution of each source, research indicates that the cane pigments make a more significant contribution to raw sugar color than do degradation reactions in processing. Farber and Carpenter compared extracts of cane juice pigments and raw sugar pigments, and showed 19 pigments from the juice went into the raw sugar, 12 pigments were in the juice but not in the raw sugar, and only 4 pigments that were in the raw sugar but not the cane juice (3). Smith and Gregory examined the nature of colorants from various

parts of the sugarcane plant, and showed significant differences in the nature of colorants in juice derived from tops and leaves, rind, and maceration (1). Smith discussed the importance of enzyme-mediated browning in sugar processing and ways to reduce it (4). Goodacre and Coombs estimated that at least half of total juice color was due to enzymatic reactions of phenolic precursors, but they did not examine the transfer of enzyme-formed colorant into raw sugar (5). Tu concluded that both the low and high molecular-weight colorants in sugar crystals originated mainly from the cane plant rather than being produced in processing under normal operating conditions (6). Workers in Australia and South Africa have shown the contribution of various agronomic factors in colorant entering the mill, including cane variety, age and maturity of the cane, presence of tops and trash, time of season (7,8). Paton concluded that high molecular weight colorants are formed initially when cane is crushed and they contribute most of the color of process streams and raw sugar (9).

This report discusses several elements of sugarcane colorants that contribute to the color of raw sugar and their importance to the raw sugar colorant complex.

MATERIALS AND METHODS

Samples. Cane juice from different varieties was obtained from the USDA Field Laboratory in Houma, Louisiana. For chromatographic separation (by GPC), cane juice was either used whole or else the polysaccharide-colorant complex was precipitated with 80% ethanol and removed by centrifugation or filtration. Colorant material from raw sugar was isolated by dialysis through regenerated cellulose bags with a nominal molecular weight cut-off of 12,000 DA.

GPC. High molecular weight components from cane juice and raw sugar were separated by gel permeation chromatography (GPC) on a 2.6 x 45 cm column, packed with Sephacryl S-500, using 0.02 M TRIS as the eluent. Elution rate was about 3 ml/min.

Colorant polymers were detected with UV detection at 214 nm, and carbohydrate polymers were detected using refractive index (RI) detection. Fractions were collected to determine the composition of individual peaks. This buffer was removed from the fractions with Rohm & Haas 118H cationic exchange resin. This was done by adding a few grams of resin to a pooled fraction and heating to about 40°C with gentle stirring until the pH was slightly below 7.0. Resin was removed by filtration. The purified fractions were concentrated by rotary evaporation and freeze dried.

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Molecular weight was determined by comparing retention time to dextran standards. The standards included sucrose (to determine permeability limit), Dextran T-10 (10,000 Da), Dextran T-500 (500,000 Da) and Dextran T-2000 (2,000,000 Da).

Hydrolysis. Ten to 20 mg of isolated material was hydrolyzed with 1 ml 2N trifluoroacetic acid (TFA) for 1 hour at 120°C. The acid was removed by rotary evaporation.

Gas Chromatography (GC). Sugars in hydrolyzed fractions were determined as their trimethylsilyl (TMS) derivatives on a 30-m capillary column coated with DB-5. The column temperature program started at 210°C for 4 min, ramped at 4°C/min until the end of the run at 12 min, when all sugars were eluted.

Mass Spectrometry (MS). Aconitic acid was identified as the TMS derivative on a Hewlett Packard 5988A GC/MS system using similar chromatographic techniques to those described above.

Determination of Leucoanthocyanins. Leucoanthocyanins (LUA) were determined in cane juice by converting them to the colored anthocyanidin during the performance of the test for total polysaccharides.⁽¹⁰⁾ During this test, after the precipitated polysaccharides have been recovered, they are solubilized by boiling in 1% sulfuric acid. This causes the pigmentation to be developed, which is then measured in a 200 ml volume at 485 nm. Results are reported as absorbance x 1000 on juice.

Isolation of Anthocyanidins. After the pigment is developed, it can be isolated from the cane juice by filtering on an Empore-SDB membrane. Sugar and salt are washed out with water, and the pigment quantitatively recovered with methanol.

RESULTS AND DISCUSSION

VHMW PEAK 1

The peak designated as VHMW (very high molecular weight) Peak 1 in earlier work on raw sugar tenates is the first peak that elutes from the Sephacryl S-500 column, indicating a very high molecular weight of at least 2,000,000 DA. It is characterized by a hazy turbidity and is present in most raw sugars (11,12,13). Of all the high molecular weight colorants, this peak has the highest crystal transfer coefficient (ie, a greater proportion is found inside the crystal than remains in the syrup phase), and it is the major, and sometimes the only, HMW peak that remains in white sugar.

Subsequently, a peak with similar characteristics was found in cane juice. The GPC chromatogram of this peak is shown in Figure 1. Isolation by GPC showed the peak also possessed a hazy turbidity. Tests for starch were negative, so the haziness was not caused by insoluble starch granules. Previous nmr (nuclear magnetic resonance) analysis at SPRI had shown that this peak isolated from white sugar consisted partially of dextran but that a small amount of another polysaccharide was present as well (13).

Cane varieties showed almost a ten-fold difference in relative concentration of this component, as summarized below:

<u>Cane Varieties</u>	<u>Relative Concentration (Normalized)</u>	
CP76-331	1.00	
CP72-370	2.46	
CP70-321	2.50	
CP85-384	5.02	
CP74-383	7.33	
CP65-357/C	9.54	C = Control
CP65-357/P	9.75	P = Treated with Polado

The peak was isolated by GPC from CP65-357 Control and Polado treated cane juice and CP72-370 Polado treated cane juice, and hydrolyzed to determine the sugar composition. Polado (N-phosphonomethyl glycine) is used as a ripening agent, or growth stimulating agent, on sugarcane grown in its second or third year (not on cane in the year of planting). The results are shown in Table 1.

The carbohydrate component of VHMW Peak 1 is about 80%. The results in Table 1 indicate that of that 80%, about half is glucose with a high proportion of arabinose and galactose, presumably as arabinogalactan, some xylose, presumably as xylan from bagasse, significant mannose, and very little or no aconitic acid.

In CP65-357, the concentration of VHMW Peak 1 did not change with treatment by the growth regulator, Polado, but the carbohydrate composition did change somewhat. Aconitic acid and rhamnose were present in the Polado-treated juice but not in the control, and there was almost a 50 percent drop in the arabinose concentration in the Polado treated sample.

Thin layer chromatography (TLC) of isolated, hydrolyzed Peak 1 showed 8 amino acids, with glycine, alanine and tyrosine being tentatively identified on the basis of their R_f values and color reactions with ninhydrin.

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The amino acid profile of hydrolyzed VHMW Peak 1 from a raw sugar tenate showed traces of amino acids in a pattern similar to that for the corresponding cane juice peak.

The sugar composition of Peak 1 from several raw sugars is shown in Table 2. It is evident, when compared to the cane juice results, that during the manufacturing process, the rhamnose, xylose and mannose-containing components were largely removed, leaving a peak containing 80-90% glucose polymer and 5-10% arabinogalactan (in an approximate ratio of 2:1 ara:gal). Nmr evidence has indicted that the glucose polymer is dextran (13).

The dextran concentration of the raws in Table 2 was: Brazil-1, 928 pm; Brazil-2, 430 ppm; Dom. Rep., 520 ppm; Australia, 287 ppm.

The composition of VHMW Peak 1 in a decolorized sugar syrup was shown to be 95% glucose with traces of arabinose and galactose. On freeze-drying, the material was pure white, but in solution, it was faintly yellow and hazy, showing that the essential hazy/turbid nature of this material had been preserved throughout the process from the cane juice all the way through to the refined product.

Removal of VHMW Peak 1. Cane juice was subjected to various treatments to determine removal of Peak 1, as measured by GPC. The results are summarized below:

1. 99% of Peak 1 was removed by room temperature filtration of cane juice through analytical grade filter aid.
2. Less than 5% was removed by hot filtration (>80°C) of cane juice through analytical grade filter aid.
3. None was removed by filtration through coarse filter paper.
4. 46% was removed by centrifuging cane juice at 12,000 RPM for 20 min.
5. 65% was removed by centrifuging cane juice at 18,000 RPM for 20 min.
6. 90% was removed by laboratory clarification with lime and heat. (Procedure: Juice was prefiltered on coarse paper, adjusted to pH 8 with lime and heated just to boiling.)
7. None was removed by acidic ion exchange resin IRA 118-H.
8. DEAE-bagasse filtration removed 95% of Peak 1.

An earlier study on the high molecular weight colorants in raw sugar showed that the VHMW Peak 1 in raw sugar (called Peak A in that study), was 10 to 50% removed by washing the raw sugar; it was removed about 88% by new carbon, 84% by new bone char, 80% by anion exchange resin IRA-900, and 81% by IRA-958. ⁽¹⁴⁾

ACONITIC ACID

During a study on the carbohydrate composition of the nondialyzable portion of raw sugar, one prominent peak appeared that could not initially be identified as a sugar (15). This peak was identified as aconitic acid by GC/MS.

The high molecular weight colorant-polysaccharide complex of several cane varieties for the presence of aconitic acid. The composition of the carbohydrate portion of the colorant-polysaccharide complex isolated from three cane varieties treated with Polado is shown in Table 3.

These results confirmed the presence of aconitic acid bound to the high molecular weight colorant complex. Aconitic acid concentration was higher in CP70-321 Control but did not change with Polado treatment, whereas it did increase with Polado treatment in the other two varieties. These results do not include the composition of VHMW Peak 1 since the isolation procedure used for this material involved filtration with analytical grade filter aid, which removes VHMW Peak 1. These results also show that sugar composition of the complex differs among varieties. For example, CP70-321 Control had a higher proportion of arabinose and galactose than the other two varieties.

The two peaks of the colorant complex in several cane juices were isolated by GPC and hydrolyzed to determine if the aconitic acid was concentrated in one peak. The results are shown in Table 4. The composition of the first peak (VHMW Peak 1), which was low in aconitic acid, was discussed in the previous section.

It is evident from the results in Table 4 that aconitic acid is concentrated in Peak 3, along with mannose. The arabino-galactan is concentrated in Peak 2.

GPC of the colorant complex of cane juice showed that the concentration of Peak 3 (peak containing aconitic acid) increased by 40% in Polado treated CP72-370 (shown in Figure 2) and 25% in CP65-357, but did not change in CP70-321 (shown in Figure 3). Although the peak amount increased by 40% in Polado treated CP72-370, Table 4 shows that the aconitic acid concentration actually decreased by 10%. The increases in peak concentration are thought to be due to

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increases in the leucoanthocyanidin colorants, as discussed in the next section. The proportion of Peak 2:Peak 3 differs considerably between the two varieties, as can be seen in Figures 2 and 3. In CP70-321, the characteristic ratio of Peak 2:Peak 3 = 18:82; the ratio for CP72-370 was 51:49.

Examination of the composition of the nondialyzable material from 10 raw sugars showed that some raws contained significant quantities of aconitic acid while others had very little (15). Examination of their GPC patterns showed that those sugars high in aconitic acid had a well developed Peak 3 in their colorant complex, whereas those that did not have very much aconitic acid had only vestigial Peak 3, with most of the color being found in Peak 2. The raws were divided into two categories: Category 1 raw sugars were high in aconitic acid and contained two colorant peaks on GPC; Category 2 raw sugars were low in aconitic acid and contained one colorant peak on GPC. These differences are demonstrated in Figure 4. Category 1 sugars contained about 6 to 10% aconitic acid in tenates; Category 2 sugars contained about 1%. The sampling of raws was too low to determine if there was a correlation to location or maturity of cane at harvest.

All the cane varieties tested from Louisiana had a peak 3 high in aconitic acid, so we do not yet know if there are varieties that lack this peak or if it decreases upon maturity of the cane.

GPC isolation of colorant peaks 2 and 3 from a Category 1 sugar and of peak 2 from a category 2 raw sugar paralleled the results for the cane juices reported above: that is, peak 3 contained a very high proportion of aconitic acid and peak 2 little or none. These results are shown in Table 5.

These results show that the colorant complex in raw sugar, as isolated by GPC has its origin in the cane juice.

Aconitic acid is the major carboxylic acid in cane juice, comprising up to 80% of total organic acids in cane juice (16). Balch, et al., found that aconitic acid was associated with less mature cane and that immature cane or cane milled with immature growing portions of stalks contained 3 to 5 times as much aconitic acid as juice from mature cane (17). Aconitic acid was in the free form in the cane juice. The current work is the first report of bound aconitic acid in cane juice.

LEUCOANTHOCYANIN

Terminology. Two types of compounds give rise to red/orange anthocyanidin compounds from colorless precursors, and this can be the source of some confusion:

Proanthocyanidin is the name given to condensed tannins or oligomers of flavan-3-ols. These can be depolymerized in acid solution to anthocyanidins (18,19).

Leucoanthocyanidin is the name given to monomeric flavonols (flavan-4-ols and flavan-3,4-diols). These are converted to anthocyanidins when heated with acid (18,19). Chlorogenic acid, luteolin and luteolinidin were identified in extracts from cane stalks infected with red rot (20). Luteolinidin was identified as the main red-orange pigment in the red rot pigment complex (20).

The presence of leucoanthocyanin (LUA) bound to sugarcane polysaccharide was first observed during the performance of the test for total polysaccharides. During this test, the alcohol-precipitated polysaccharide is redissolved in boiling 1% sulfuric acid. At this time, it was noted that many sugarcane juices developed a pink to orange color. The development of pigment in hot acid is the classic test for the presence of leucoanthocyanidins.

The LUA in 10 cane varieties from Louisiana was determined in 1990 and is shown in Figure 5 (21). It can be seen that there are marked varietal differences in the concentration of LUA. In subsequent years, tests for LUA in several of these varieties has shown LUA varietal concentration to be quite consistent. Thus, it appears that CP72-370 is a high-LUA variety and CP70-321 is a low-LUA variety.

The leucoanthocyanin attached to polysaccharide in 3 cane varieties was determined in plants that had been treated with the growth regulator, Polado, and compared to plants that had not been treated (controls). The results are shown in Table 6.

As with the presence of aconitic acid in the colorant complex, we wished to determine if the LUA was concentrated in one or both of the colorant complex peaks. Isolation of the peaks by GPC and subsequent hot acid treatment showed that the main pigment-producing peak was GPC peak 3, the same one that contained aconitic acid. The increase in LUA concentration with Polado treatment of CP72-370 (Table 6) coincided with the increase in Peak 3 noted in CP72-370 (Figure 3).

The absorbance maximum of the isolated LUA in methanol was 490 nm, very close to that of luteolinidin, the leucoanthocyanin that is associated with red rot disease of sugarcane.

While this study has concentrated on the LUA associated with the high molecular weight polysaccharide, some cane varieties also contain large amounts of "low molecular weight" leucoanthocyanins - that is, LUA that is present in cane juice after the high

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molecular weight fraction has been removed by precipitation with 80% ethanol.

The tremendous reservoir of potential red pigments that exists in sugarcane tissues as the colorless leuco-compound could provide and explanation for the large quantities of red pigment produced by the sugarcane plant under stress, either by disease or environmental conditions. The pigments are released by acidic conditions, which occur in various disease states. (22,23)

The anthocyanin can be quantitatively isolated from sugarcane by use of Empore-DVB membrane. (DVB = divinyl benzene, similar to the composition of the adsorbent resin XAD-2.)

LUA in raw sugar color. It was of interest to note whether any of the LUA carried over from the cane juice into the HMW colorant complex of raw sugar. Several experiments confirmed that LUA did occur in some, but not all, raw sugar HMW colorant. Hot acid treatment of the tenates of several raw sugars produced varying degrees of red color reaction, shown in Table 7.

Isolation by GPC of individual colorant peaks from several raw sugars, with subsequent hot acid treatment (during hydrolysis) showed that the LUA pigment was also concentrated in Peak 3, as it was in cane juice. This pigment could be isolated using Empore-DVB membranes in a manner similar to the isolation of pigment from cane juice.

Anthocyanins in the free state in cane juice are unstable in alkaline conditions and easily removed during clarification. However, it is possible that the bound, colorless form, reported in this study, is more protected from removal and can continue into the raw sugar. There are, however, no indications of its presence in refined sugar.

SUMMARY AND CONCLUSIONS

Table 8 gives a brief overview of the characteristics of the three high molecular weight colorants discussed in this report.

VHMW Peak 1. In an earlier study of the non-dialyzable components in refining processes, refined sugar was noted to have one major very high molecular weight peak (11). Even in very low-color refined sugars, this peak, mostly polysaccharide, was present. Nmr confirmed its essential polysaccharide nature (13). The work reported in this study has traced the origin of this peak to the cane juice and has further elucidated its composition. In sugarcane juice, VHMW Peak 1 is about 80% carbohydrate with some protein, and a hazy character; the molecular weight is at least

2,000,000 Da. The carbohydrate portion is composed of about 50% glucose, 10-20% arabinogalactan, little or no aconitic acid, about 6% mannose, and 2-4% xylose. There are strong varietal differences in the concentration of this component. In the raw sugar, the carbohydrate proportion increases to about 90%, with most of the xylose, rhamnose and mannose being removed, indicating possible heterogeneity in the peak. A small amount of protein remains in VHMW Peak 1 in raw sugar. The fine, hazy turbidity of this peak carries through most purification processes and is the major high molecular weight colorant in refined sugar.

Aconitic Acid. Aconitic acid was found to be concentrated in one high molecular weight peak (Peak 3) that appears in the colorant complex of both cane juice and raw sugar. In both cane juice and raw sugar, aconitic acid comprises about 30% of the hydrolyzable portion of Peak 3. The molecular weight of this component is calculated to be around 300,000 Da. Peak 3 is about 50% carbohydrate, the rest being made up of uncharacterized colorant polymers containing bound leucoanthocyanin, as described above. This is the first time aconitic acid bound to HMW colorant-polysaccharide complex has been reported.

Leucoanthocyanins. Colorless or yellow leucoanthocyanin (LUA) precursors were found to be present in Peak 3 of the HMW colorant of cane juice. Concentration was highly dependent on variety and increased in some varieties when treated with the growth regulator, Polado. The bound LUA carried over into many raw sugars.

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Table 1. Composition of sugars in VHMW Peak 1 from cane juice.

	CP65-357	CP65-357	CP72-370
Sugar	Control	Polado	Polado
arabinose	8.1	4.4	18.5
rhamnose	0	1.4	0
xylose	2.4	3.2	9.8
mannose	6.5	7.0	5.6
galactose	7.2	6.8	4.6
glucose	50.5	47.2	49.0
aconitic acid	0	2.7	0

Table 2. Sugar composition of VHMW Peak 1 from raw sugars.

Sugar	Brazil-1	Brazil-2	Dom. Rep.	Australia
arabinose	3.6	6.1	7.5	1.4
rhamnose	0	0.8	0	0.1
xylose	0	2.0	0	1.1
mannose	0	0	0	0.3
galactose	1.1	3.5	2.8	0.6
glucose	81.5	83.2	85.9	93.0
aconitic acid	0	0	0	0.6

Table 3. Monosaccharide and aconitic acid composition of isolated high molecular weight colorant-polysaccharide complex from cane juice.

Variety	CP65-357		CP70-321		CP72-370	
Sugar	Control	Polado	Control	Polado	Control	Polado
ara	4.9	4.1	12.2	11.0	7.5	13.5
rha	0.4	0.1	0.8	0.7	0.8	0.3
xyl	1.0	1.3	1.6	2.1	1.0	7.1
man	6.6	9.6	6.3	5.1	14.7	9.1
gal	2.7	2.0	7.4	7.1	5.4	4.5
glc	69.8	66.2	49.9	51.6	51.3	46.8
Aconitic	5.3	7.3	11.4	11.7	9.4	11.2

Table 4. Monosaccharide and aconitic acid composition of HMW colorant peaks from cane varieties.

Variety	CP72-370/Control		CP72-370/Polado		CP70-321/Polado	
Sugar	Peak 2	Peak 3	Peak 2	Peak 3	Peak 2	Peak 3
ara	13.5	0.6	7.6	6.4	29.3	2.9
rha	0.8	0	0.2	0.2	1.8	0.5
xyl	0.7	0.4	4.4	4.0	5.7	1.0
man	10.8	29.5	8.0	23.6	6.1	29.3
gal	6.2	1.5	2.5	1.3	13.9	3.9
glc	42.6	26.1	55.9	34.2	18.9	13.0
Aconitic	4.0	31.7	0	21.2	1.2	35.1

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Table 5. Composition of HMW colorants from raw sugars.

Sugar	Category 1 Sugar		Category 2 Sugar
	Peak 2	Peak 3	Peak 2
arabinose	15.5	2.0	13.3
rhamnose	1.0	0	1.2
xylose	7.5	3.5	10.1
mannose	2.0	0	5.4
galactose	23.9	0	4.1
glucose	42.7	49.6	55.4
aconitic acid	0	31.7	3.0

Table 6. LUA concentration in cane varieties treated with Polado.

Variety	Control	Polado	% Increase
CP72-370	71	115	62
CP65-370	42	59	40
CP70-321	18	19	6

Table 7. Presence of LUA pigment in HMW raw sugar colorant.

<u>Sugar</u>	<u>LUA Color Reaction</u>
Louisiana, 601	Moderate
Louisiana WRS	Faint
Louisiana, C	Strong
Louisiana, A	None
Florida, 1	None
Florida, 2	None
Hawaii, 89	Strong
African, 507	Moderate
Queensland	Faint
Peru	Strong
Philippines	None
Nicaragua	Already red colored, no more rxn.
Brazil	Strong
Panama	None
Ivory Coast	Faint
Dom. Rep.	Moderate
Trinidad	Moderate

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Table 8. Overview of HMW colorants in cane processing

HMW COLORANTS IN CANE JUICE

Peak 1

In all cane varieties tested in Louisiana
MW about 2,000,000 Da
Pale yellow to straw color
Contains glucose, arabinogalactan, xylose and mannose
Consists of about 80% carbohydrate
Contains some protein
Composition may change with Polado treatment
9-fold concentration difference in varieties
Has a fine, hazy turbidity
Highest crystal transfer coefficient

Peak 2

In all cane varieties tested in Louisiana
MW about 500,000 Da
Color is brown or yellow-brown
Contains 50-60% carbohydrate
High in arabinose, galactose, mannose, glucose
Contains small amounts of leucoanthocyanidin
Contains up to 4% aconitic acid, but usually less
A major colorant

Peak 3

In all cane varieties tested in Louisiana
MW about 300,000 Da
Color is dark brown
Contains about 50-60% carbohydrate
Very high mannose content
Very high aconitic acid content
Contains bulk of the leucoanthocyanidin pigment
Concentration is very susceptible to Polado treatment in some,
but not all varieties, increasing 40-60%
Varieties have characteristic ratios of peak 2:peak 3
A major colorant

HMW COLORANTS IN RAW SUGAR

Peak 1

Always present in raw sugar
MW about 2,000,000
Pale yellow color
Mostly glucan with some arabinogalactan
Contains small amount of protein
Carbohydrate content is increased to 90% compared to juice
Contributes a small amount of turbidity

(Table 8, continued)

Peak 2

Present in all raw sugars

MW about 500,000 Da

High in glucose, arabinose and galactose

The mannose portion from the cane juice is usually absent

A major brown colorant

Peak 3

Absent in Category 2 raw sugars

MW about 300,000 Da

Contains aconitic acid and leucoanthocyanidin

Easily removed by anion exchange resin due to acid nature

A major dark brown colorant

HMW COLORANTS IN WHITE SUGAR

Peak 1

Almost always present in white sugar

MW about 2,000,000

Colorless to pale yellow

About 95% carbohydrate

Mostly glucose

Contains turbidity

Nmr indicates presence of dextran and another polysaccharide

Peaks 2 and 3

Usually absent or insignificant

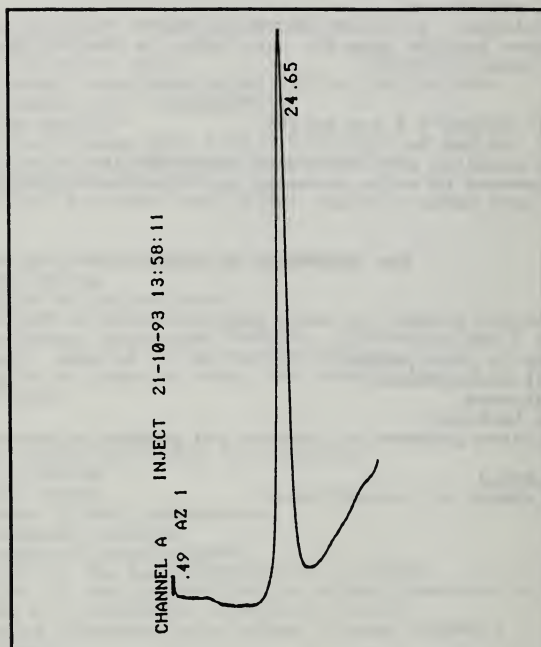


Figure 1. VHMW Peak 1 in cane juice from variety CP72-370.

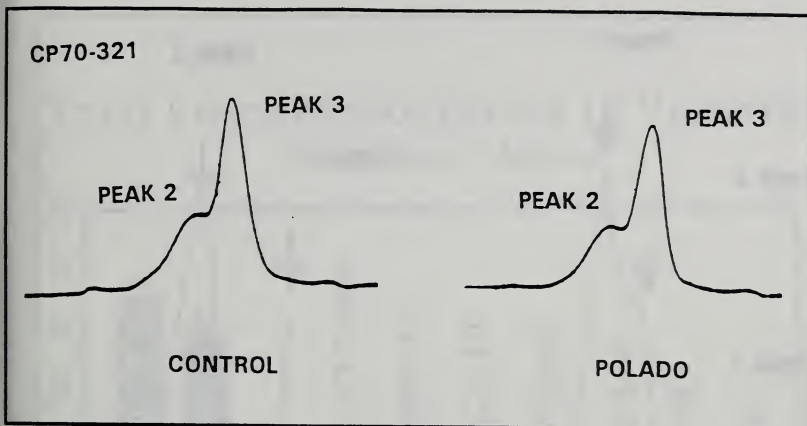


Figure 2. GPC of colorant complex from CP70-321 cane juice.

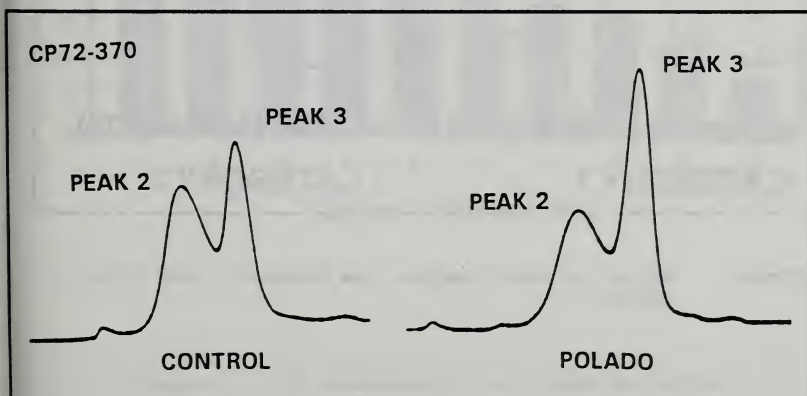


Figure 3. GPC of colorant complex from CP72-370 cane juice.

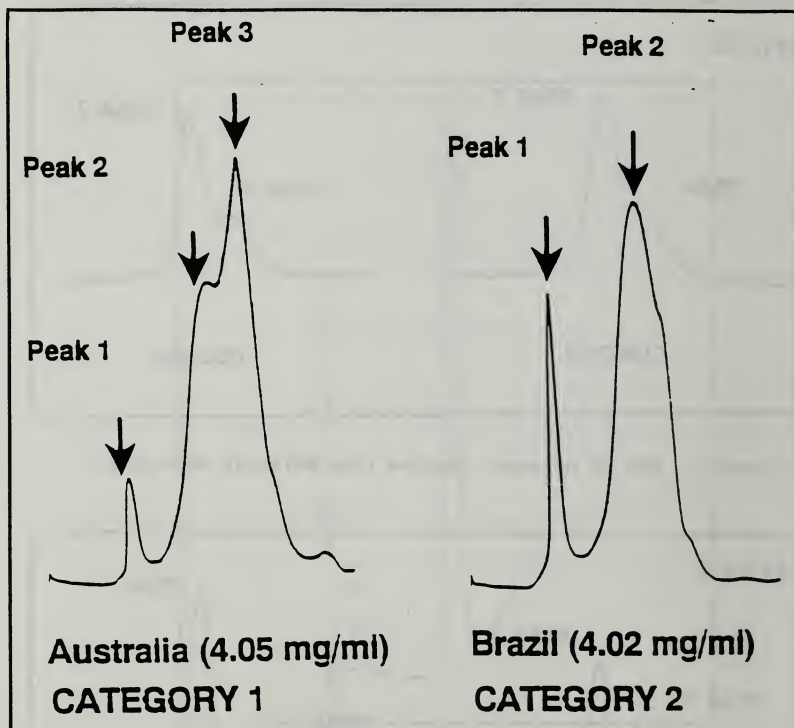


Figure 4. GPC of colorant complex from Category 1 and 2 raw sugars.

Total Leucoanthocyanins in Varieties Seasonal Means

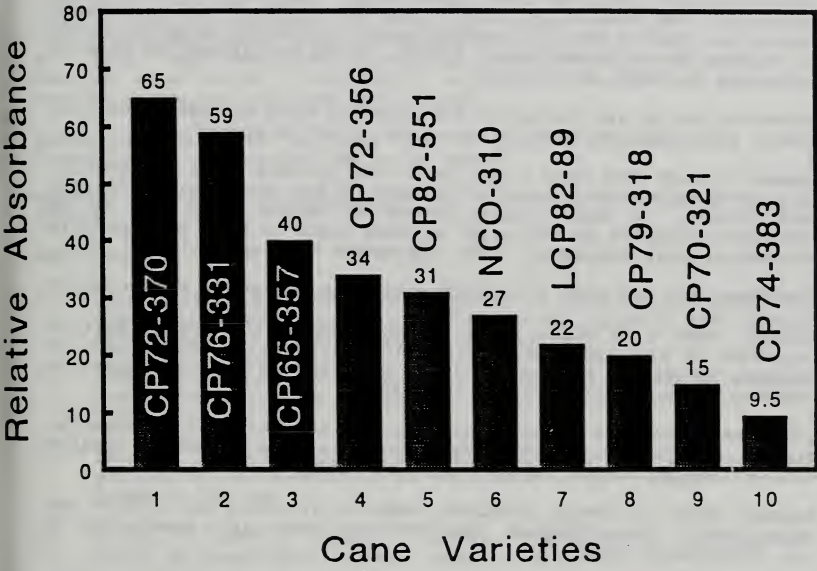


Figure 5. LUA concentration in 10 cane varieties.

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DISCUSSION (from questions received by correspondence, after the conference).

Question: Since VHMW Peak 1 is a mixture, can it be resolved by using a different chromatographic system?

Answer: Very few chromatographic media exceed the very high molecular weight separation range of Sephacryl S-500. In previous work, when we used only water as the eluent solvent, this peak did partially resolve into two components, but the columns were unstable. The TRIS buffer stabilizes the column but also reduces resolution of VHMW Peak 1. Another possible chromatographic medium to explore would be Sephacryl S-1000, which is claimed to resolve molecules as large as viruses.

Question: How do you reconcile the relative ease of removal of VHMW Peak 1 with the fact that it persists in white sugar?

Answer: Enough VHMW Peak 1 remains in the process to be concentrated in the white sugar, especially since it has such a high transfer coefficient. A much larger amount of white sugar has to be extracted relative to raw sugar or cane juice to give a comparable chromatographic response. The components are very soluble.

Question: Are all Peak I constituents transferred to the crystal?

Answer: Peak 1 in white sugar is enriched in the glucan portion compared to raw sugar and cane juice, indicating that there is unequal transfer. However, this is complicated by its removal in various steps in the refining process.

Question: Could the apparent increase in aconitic acid concentration in colorant-polysaccharide complex in cane varieties treated with Polado be due to natural variability?

Answer: This is always possible, especially since our sample was quite small. Nevertheless, the varieties were quite consistent in the differences observed.

Question: You indicate that condensed tannins can be depolymerized to anthocyanidins. It is my understanding that condensation of tannins is an oxidative polymerization that is not readily reversible to labile monomers.

Answer: Tannin is a collective term referring to polyphenolic molecules with the ability to precipitate protein. Various groups of compounds with different precursors fall into this classification. The literature rather consistently states that condensed tannins are oligomers of flavan-3-4-diols and flavan-3-ols that are hydrolyzable with strong mineral acid (1).

A review states, "Tannins are defined as high molecular weight polyphenolic compounds that have the ability to bind with protein and preserve animal hides. However, the term 'tannins' is commonly used to refer to polyphenolic compounds." (2)

Question: Do you mean to imply that chlorogenic acid and luteolin are not natural components of healthy cane?

Answer: Not necessarily. Chlorogenic acid is very common in many plants, and has reported previously in sugarcane (3,4). The study referenced used thin layer chromatography for detection, which may not have been sensitive enough to detect these components in healthy cane juice. Chlorogenic acid and luteolin did increase during infection with red rot disease.

Question: Could the aconitic acid be removed by base--ie, is it bound to the polysaccharide as an ester?

Answer: That is an experiment we will have to conduct in the future.

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FIBER IN SUGARCANE AND BAGASSE

Margaret A. Clarke¹, Benjamin L. Legendre², Raul Perdomo³,
Les A. Edye¹, Rebeca S. Blanco¹, Xavier M. Miranda¹, and Angel K.
Kelly¹

¹Sugar Processing Research Institute, Inc., New Orleans, Louisiana,
USA

²Sugarcane Research Unit, Agricultural Research Service, U.S.
Department of Agriculture, Houma, Louisiana, USA

³Okeelanta Corporation, South Bay, Florida, USA

ABSTRACT

The analysis of fiber in sugarcane, whether for development of new varieties, for crop payment calculations or for factory process control, is a tedious and time-consuming procedure when run directly. For these reasons, indirect measurements of fiber have been developed for routine analysis.

In work on development of a Near Infrared (NIR) calibration for fiber, inconsistencies in the indirect measurements made them unsuitable for calibrations. Direct measurement of fiber, in both whole sugarcane and mill-run bagasse appeared necessary.

Various direct and indirect analyses for fiber are discussed herein, and progress on development of an NIR analysis for direct fiber determination is reported.

INTRODUCTION

The fiber content of sugarcane has always been an important component in sugarcane breeding. Fiber is composed of cellulose and hemicellulose with shorter fibers in the central pith section of the stalk, and longer fibers in the section between the central zone and the outer stalk coating. Historically, high fiber cane has been associated with low sucrose yields, though this association is not always the case. High fiber canes have been selected for areas where there can be high winds and heavy weather, to produce cane that will remain standing in the field without lodging or stalk breakage. However, higher fiber levels in cane produce more bagasse (the residual fiber after cane is ground and sugarcane juice extracted), and consequently can, in milling, require large amounts of water of maceration to be added, and incur higher losses

of sucrose, retained in bagasse, than do low fiber canes. The additional water increases energy demand in the boiling house. Higher fiber canes wear down mill rolls more rapidly. Conversely, where more bagasse is desirable, that is, where bagasse in excess of the normal requirement to fuel the factory is used as a byproduct e.g. for paper, board or furfural manufacture, high fiber cane is in demand. The current increase in use of excess bagasse for cogeneration of electricity in the factory power plant, for sale to the local grid, is increasing the demand for high fiber cane, to provide more raw material for electricity production. This, in turn, has increased interest in analysis of fiber in cane, to provide an accurate assessment of raw material for cost analysis.

Where sugarcane is purchased on quality factors (6), fiber is a factor, with higher fiber values varying directly with lower juice % cane, and lower commercial recoverable sugar (C.R.S.).

Traditional analytical procedures. Fiber can be determined directly, by washing ground cane in a tared bag, drying to constant weight, and stating the weight of dry material as fiber content (7). This method is seldom practiced other than in cane variety development programs, where cane is hand-cleaned and free of tops, trash, and mud. Fiber is generally estimated or calculated by difference between cane weight and juice weight, or with factors for juice solids, extraction, and trash. Moisture is determined directly by oven drying. An example is the calculation used in the core/press method for cane sampling in Louisiana, where "residue" is the pressed material remaining after juice is extracted from the cane sample (6).

$$\text{Fiber \% Residue} = 100 - \frac{\text{moisture \% residue}}{(1 - \text{extracted juice Brix}/100)}$$

$$\text{Fiber \% cane} = \frac{(\text{fiber \% residue}) (\text{total residue/cane})}{100}$$

The so-called fiber, in an actual field cane sample, not a hand-cleaned variety test sample, is really true fiber plus all other non-cane solids, i.e., fiber plus mud, leaves, tops, and trash. This difference increases in importance in areas where cane is harvested immature or is unburned, or in rainy, muddy weather. Cane is analyzed for moisture by drying the residue after the juice has been extracted by a hydraulic press, and for fiber by a calculation on dried weight of residue less residual Brix. In areas where cane quality is measured by other than the core/press method, a calculation by difference is made, sometimes combined with the amount of bagasse produced.

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In bagasse analysis, bagasse itself (which leaves the mill at 47%-55% moisture) is seldom weighed directly. Weight is usually calculated by difference and by prediction on cane "fiber". Bagasse is analyzed for moisture by drying, and for sucrose by an aqueous extraction procedure (in Waring-type Blendor, with pol analysis of the measured water phase (8)). There is considerable variation in methodology among factories with regard to weight of sample and time of drying or extraction.

NIR analysis. The purpose of this work is to investigate analysis of sugarcane fiber and bagasse by near infrared techniques, and to augment or replace current analytical methods. Work on analysis of sugarcane by NIR was initiated by Sverzut (11), at the Southern Regional Research Center with promising results. Vaccari and Mantovani (12) first introduced NIR into sugar factories in 1987. Berding and coworkers (2, 3) in Australia have made detailed studies of NIR analysis of fibrated cane and expressed juice for cane breeding studies for fiber, moisture, Brix, pol, purity, conductivity and CCS (commercial cane sugar). Berding's initial work (2, 3) employed a filter-type NIR spectrophotometer. In more recent work (1), he has used a scanning instrument with more satisfactory results. Berding indicates that his results on reflectance spectroscopy of fibrated cane were still not equivalent to laboratory results in reproductibility and repeatability. The statistical ranges of laboratory test error quoted by Berding were from tests on a small set of hand-cleaned sugarcane and were more precise than those found on larger sets of hand-cleaned cane (see Results section), and certainly less variable than ranges for factory cane. Another factor in the difference between Berding's results and the current study, may be that Berding et al. (2, 3) used a model NR 7080 sample holder, which has a smaller sample cell window than that used in work reported herein.

Meyer (9, 10) in South Africa reported NIR work on cane juice and other materials using filter-type instruments, and more recently has used scanning NIR on shredded cane and other substrates. He reports the possibility of NIR as a screening tool for disease resistance (10). Clarke and Edye reported promising preliminary results on analysis of factory bagasse (4).

METHODS AND MATERIALS

NIR analyses. NIRSystems Model 6500 scanning NIR spectrophotometer (NIRSystems, Inc.) was used, with Sample Transport Module, and a large cell for coarse samples, with about 60 cm² surface area of sample, for bagasse and cane fiber samples. Samples were all measured in reflectance, from 1100-2500 nm, using the lead sulfide detector, at an average of 50 scans per minute. A table top or

notebook computer was used to store and handle data. Several different models were used at the various locations. NIRSystems "NSAS" or "ISI" software packages were used for all calibrations and predictions (analyses). Calibrations were developed using "conventional" NIR technology procedures, including selection of optimum wavelengths for log 1/R, and first and second derivatives thereof by forward stepwise regression.

Laboratory analyses. Laboratory analyses were conducted by standard factory procedures (6, 7) at sugarcane factories and USDA research facilities in Louisiana and Florida. Direct fiber analysis was made by washing cane in a tared bag and drying to constant weight (2, 7). A wet disintegrator method, without a bag, is used in Australia and South Africa (5). All samples were analyzed by NIR fresh on site. For testing at SPRI, samples were then frozen and shipped, in frozen state, to SPRI's labs.

RESULTS AND DISCUSSION

Fiber in sugarcane. Trials on whole cane began in 1992, with tests run on an NIRSystems Model 6500, in reflectance mode, using a "coarse cell" with quartz plate and 60 cm² area exposed to the analysis beam. Whole chopped cane, taken off the first mill (crusher) in a mill tandem without a shredder, proved too coarse and too variable in sample size for accurate analysis for fiber. Samples, shipped to SPRI, were analyzed directly for fiber. Using any available statistical package, it did not appear possible to obtain a correction coefficient better than $r=0.8$, and errors of calibration and of prediction were greater than 1.5%.

The next step was analysis of cane from a prebreaker grinder, both at a factory core/press lab, and at an experimental station. This cane, being in smaller pieces than cane taken directly from the crusher, gave better results: reasonable associations were obtained for moisture from factory lab samples, ($r=0.9$), but errors of prediction were still high.

The question of comparing data from clean cane with data on factory cane containing mud and trash remained. It is proposed that calibrations developed for fiber, moisture, pol, and Brix on clean cane will hold, when validated, for factory cane. The mud and trash can then be determined by difference. It is logical to have direct analysis for the products (sugar and fiber) and indirect analysis for extraneous material.

In 1993, trials began on cane samples prepared in a Jeffco cutter-shredder. These samples yielded NIR results (Table 1) within ranges of error for normal laboratory methods on cane from a Jeffco

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cutter-shredder (Table 2). Apparently the smaller, more uniform, particle size has improved the accuracy and precision of results. Fiber was calculated according to the procedure outlined by Legendre (6) and was also determined directly on a set of cane samples ($n=47$), frozen and shipped to SPRI.

The cane samples were chosen to reflect the widest range of fiber content found in Florida sugarcane and encompassed the range found for most other varieties ground in other states and/or countries. Because these varieties were in a variety test program, cane was clean and stripped of leaves and trash before milling. The values for direct determination and calculated determination of fiber were quite similar although with little correlation to one another. The correlation coefficient between the two analyses was only $r=0.73$, but the ranges of values (Table 2) were very similar, and the average value for calculated fiber was 13.32% (S.D.=2.79) compared to average value for directly determined fiber of 12.77% (S.D.=2.4-7). There can be errors in the calculated value from extraneous material remaining on cane (probably why average calculated values were higher, although only by 4%) as well as in the moisture determinations; the direct determination suffered from errors in moisture determination, taring the bag, and weighing.

Correlations among laboratory determined values for sugarcane processed in the Jeffco cutter-shredder are shown in Table 3.

Results certainly merit further testing. Tests are planned on further variety program samples, and on factory cane prepared in a Jeffco cutter-shredder. The potential use of one or two NIR spectrometers with operators, in lieu of equipment for oven drying, pol, and Brix, and the potential addition of true (HPLC) sucrose to the measurements provides a strong incentive for further work.

Bagasse analysis. Bagasse analyses by NIR were made at three sugarcane factories, again using a coarse sample cell (60 cm² surface), reflectance mode, and range of 1100-2500 nm. Results, ranges and standard deviation on the factory laboratories own measurements for pol and moisture are shown in Table 4, and from NIR analyses in Table 5. Calibration curves are used as models to predict pol and moisture values. On the separate sets of results from each factory, calibration curves developed for a factory could be used to predict values for that factory, with results within the standard deviation of the comparable laboratory measurement. But calibrations from one factory could not be satisfactorily used for another factory, nor could the three sets be combined into a useful universal calibration curve - the errors were too great. It is probable that differences in laboratory analyses used at the three factories account for the incompatibility of the results, because NIR spectra of the three sets can be combined - there is no

difference in spectral characterization among the three sets of bagasse samples.

Future work will require analysis of bagasse from several sources by a common technique, probably at SPRI on frozen samples.

SUMMARY AND CONCLUSIONS

Trials on developing NIR analysis of whole sugarcane for fiber, moisture (also Brix, pol, purity) have shown that cane must be finely shredded (as from a Jeffco cutter-shredder) to obtain good analyses. Results on finely shredded cane are then within normal laboratory error, both for calibration and prediction, for fiber (calculated or direct determination), moisture, Brix and pol. Purity is better calculated from pol and Brix than read directly. The NIR methodology offers the possibility of including analyses for true sucrose (chromatographic) and dextran in the direct NIR analysis.

Bagasse can be analyzed by NIR for moisture and bagasse, within normal laboratory error, and at much greater speed and frequency than is available with current tests. Results on bagasse pol and moisture can be obtained within a few minutes of sampling, so that mill tandems can be adjusted immediately for maximum extraction. However, it appears necessary at this time to construct a specific calibration for each factory; universal calibrations for bagasse are not yet available.

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Table 1. NIR measurements on shredded cane.

Components	Correlation coefficient	Std. error of prediction
Calibration		
Brix	0.99	0.30
Pol	0.98	0.46
Purity	0.94	2.31
Moisture	0.91	1.26
Fiber (calc.)	0.99	0.38
Fiber (weight)	0.95	0.74
T.R.S. ^{1/}	0.98	10.7

n = 47

Validation		
Brix	0.96	0.54
Pol	0.96	0.78
Purity	0.91	3.18
Moisture	0.82	1.65
Fiber (calc.)	0.97	0.64
Fiber (weight)	0.97	0.60
T.R.S.	0.96	8.67

n = 20

^{1/} T.R.S. = Yield of theoretical recoverable sugar per ton of cane.

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Table 2. Statistics of laboratory measurements.

	Brix	Pol	Purity	Fiber ^{1/} (calc.) (on cane)	Moisture ^{2/}	TRS ^{3/}	Fiber ^{4/} (weight)
Max. value	20.46	18.96	92.66	17.72	66.67	294.82	17.56
Min. value	13.72	9.06	65.10	8.43	42.64	103.76	8.94
Average	17.31	14.10	81.14	13.32	59.53	210.98	12.77
Std. dev.	1.87	2.50	6.67	2.79	4.24	54.83	2.47

^{1/} Fiber (calc) is calculated from press cake weight, and moisture, with a Brix adjustment factor.

^{2/} Moisture is calculated from whole and dried weight of press cake.

^{3/} TRS = Theoretical recoverable sugars, calculated, as are the other factors, by the procedures of Legendre (6).

^{4/} Fiber (weight) is determined experimentally (see Methods and Materials).

Table 3. Correlations among laboratory measurements.

Comparisons	Correlation coefficients
Calculated fiber and weighed fiber:	0.73
Calculated fiber and moisture:	0.78
True fiber and moisture:	0.62
Total recoverable sugars and purity:	0.87

Table 4. Statistics of laboratory measurements on bagasse.

Factory	n	Moisture			Pol		
		Maximum	Minimum	S.D. ^{1/}	Maximum	Minimum	S.D. ^{1/}
A	75	58.0	54.0	1.50	2.88	2.07	0.26
B	95	58.9	50.1	1.70	3.26	1.76	0.31
C	45	56.0	50.0	1.99	3.55	2.36	0.30

^{1/} S.D. = Standard deviation.

Table 5. NIR analysis of bagasse.

Factory	Pol			Moisture		
	Correl. coeff.	Standard error of calib.	Standard error of prediction	Correl. coeff.	Standard error of calib.	Standard error of prediction
A	0.80	0.10	0.11	0.74	1.08	1.00
B	0.90	0.12	0.38	0.87	0.77	1.39
C	0.75	0.33	0.33	0.82	1.12	1.20

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DISCUSSION

Question: It's enjoyable to hear such a well put-together presentation. We hope that one day we'll have the same insight on sugarbeet fiber that we have on cane fiber. We've heard a lot today about NIR - would you comment on when NIR might be expected to be a commercial system for analysis of sugarcane.

Clarke: Yes, thank you for your kind words. It is possible that this system could be commercial a year from now. We are hoping to put a scanning NIR in a cane factory core laboratory, for juice analysis and cane analysis for an entire season, beginning in September this year. If the comparison with regular commercial analysis shows good results, then the NIR system can become commercial next year.

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EXPERIENCE OF START-UP AND OPERATION OF AN AUTOMATIC ROBOT STATION FOR SUGAR ANALYSES

Soili Ylisuutari

Finnsugar Sucros Ltd, Kantvik, Finland

ABSTRACT

In the spring of 1993 a laboratory robot for basis analyses was validated at Finnsugar Sucros Ltd. Porkkala Refinery. Three-shift operation of the new system was implemented in May 1993.

Dry substance, colour, conductivity ash, and invert sugar of process liquids and liquid sugars are determined, as well as moisture, invert sugar and ash of white sugars. The robot is operated by the staff of the Quality Control Laboratory. Approximately 50 to 80 analyses are carried out in every shift.

The efficiency and capacity of the robotic system have met with expectations. Before starting to run the system independently, each operator experienced a ten-day training period led by a senior analyst.

Due to the pioneer nature of the system, more frailties than expected have appeared. Mechanical construction and sensors of the auxiliary devices have been one main source of defects. Some disturbances have occurred in the data communication between the work station PC and the measuring instruments. During the first two months, operation was interrupted by error in the system approximately per shift. The frequency of these situations has now been reduced to less than one a day. Few of the errors proved to be user errors.

Goals and advantages of this system seem to be similar to those of NIR-methods, such as efficiency, minimal sample preparation and application of advanced software in judgment of the results, as well as flexibility to monitor several characteristics of a sample simultaneously. The main difference is in the basics of the methods of analysis: the robot makes use of traditional methods, many of them ICUMSA based, while the aim of NIR analysis is to develop and validate new procedures.

The system will be improved this year. Methods for analyzing sugar syrups and samples from the recovery station of the refinery will be developed and validated.

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INTRODUCTION

In the spring of 1993 a laboratory robot for basic analyses was validated at Finnsugar, Sucros Ltd Porkkala Refinery. Three-shift operation of the new system was implemented in May 1993.

Dry substance, colour, conductivity ash and invert sugar of process liquids and liquid sugars are determined, as well as moisture, invert sugar and ash of white sugars. The robot is operated by the staff of the Quality Control Laboratory. Approximately 50 to 80 analyses are carried out in every shift.

Goals and advantages of this system are efficiency, minimal sample preparation, flexibility to monitor several characteristics of a sample simultaneously and application of advanced software in judgment of the results.

STRUCTURE OF THE SYSTEM

Construction and equipment

The system consists of the following items:

1. Workstation-PC, which provides functions such as
 - graphical user interface
 - sample preparation and sample data
 - analytical procedures
 - communication with the measuring instruments
 - communication with the LIMS-system data base
2. Robot controller
 - controls the auxiliary devices using a programmable logic controller (PLC) as an interface; the number of I/O can be as high as 100
 - controls the robot manipulator
3. Robot manipulator, an industrial 4-axis SCARA-type manipulator, grippers for sample cups, syringes and moisture analyses dishes
 - moves the cups, syringes and dishes to and from the measuring instruments and auxiliary devices
4. Auxiliary devices
 - mixers
 - dosing of syringes
 - feeders, elevators, conveyors
 - lid opener
 - etc.

The workstation PC communicates with the measuring instruments bi-directionally taking the program parameters to the instruments and receiving measurement data as well as error signals. It also controls the state of the auxiliary devices.

Figures 1 and 2 show the items of equipment in the system.

Principles of operation

Process operators take the samples marked with identification data to the laboratory. There is a regular analyzing program for process control and product quality control. For extra analyses or extra samples a requirement form is filled.

Operation of the robot system belongs to the routine of an analyst, who also performs manual refinery analyses simultaneously. The system is operated in three shifts.

The operator enters the sample into the system. The sample is logged into the workstation-PC: after a password, the sample identification is entered and the necessary analyses are chosen. Sugar samples are delivered pre-weighed to the robot station in sample cups, one cup for each analysis. Liquid samples are delivered in syringes; the same syringe is used for all the analyses. Samples are marked with a bar code label.

The sample is taken by the robot manipulator to the bar code decoder for identification. The sample cups are chosen sequentially to optimize the use of measuring instruments. A short description of the steps of measuring sequences is given in Table 2.

Results of each measurement are transferred to the workstation-PC; the final results are calculated, printed and transferred to the laboratory information system data base.

VALIDATION AND START-UP

Analytical methods

The structure of the system, the measuring instruments, auxiliary devices and analytical sequences are designed to perform the following analyses:

- dry substance of process liquids by density measurement
- moisture of sugars by loss on drying
- colour of both sugars and liquids by spectrophotometric method
- invert sugar of both sugars and liquids by enzymatic method

Table 1 describes the principles of the methods.

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Validation of methods

Before implementing as routine operation the computer system, data communication routines and analytical procedures were tested and documented. Precision and reproducibility of unit operations, such as weighing and dosing, were tested.

Sensitivity, linearity and repeatability of the measuring instruments were tested. Precision of the methods was compared with those of the earlier methods (see (Table 3) and the correlation between the traditional system and the robot methods was calculated.

During this validation procedure all new methods were found to match to the given limits with certain restrictions.

The application area of the enzymatic invert sugar method is no longer linear if the invert sugar level exceeds 1%. With this restriction the method is suitable for process liquors and white sugar samples.

There was a need to apply the Bottlers method for colour additionally for process liquors. With the original calculation of turbidity at 720 nm the final results became negative. Due to good correlation with the ICUMSA method it was decided to develop the calculation of final results so that we got a proper method for all-day process control purposes.

The correlation between loss-on-drying methods was not very good. This is mainly due to poor repeatability of Sucros' old method, where a drying oven of low temperature was used. The repeatability of the new IR-moisture analyzer was twice as good as that of the previous loss-on-drying method.

Testing of the computer system

The programs of the workstation-PC and robot controller were tested. The documentation was thoroughly checked and found to be correct and sufficient for later operation, fault detection and maintenance purposes. Some of the possible system error situations were simulated to see how these would affect on the programs.

Operator training

Two laboratory analysts took part in the development work during all the projects. Later these persons were responsible for user guidance and user instructions. The other analysts experienced a two-week training period before independent operation.

Maintenance of the system is mainly taken care of by the instrument and electrical staff of Porkkala plant. These people were made familiar with the system on a two-day course held by the robot mechanic suppliers and computer programmers.

EXPERIENCE OF OPERATION

Number of samples analyzed

During the first year of operation approximately 50-80 samples were analyzed in every shift. For each sample three to four analyses were determined. The capacity of the system was found to be well within expectations. It takes only one to two hours to analyse this amount of samples, and requires 20-30% of the capacity available. Further it takes one hour in a shift to enter the samples into the system. Compared with the time that the manual analyzing would require, the goal to relieve workers is achieved.

Reliability and failure frequency

Due to the pioneer nature of the system more frailties than expected have appeared. During the first months of operation was interrupted due to malfunction of the system approximately once in a shift. The number of interruptions has now been reduced to less than once-in-a-day level.

Most of the defects are communication errors between a measuring instrument and the workstation PC. These faults are not of a serious nature. They can quickly be recovered from by shutting off the instrument and resetting the measuring sequence. Fault classification is outlined in Table 4.

Equally common but more disturbing are flaws in the mechanics and devices. Even though the devices (the machinery?) were carefully designed and thoroughly tested, wearing, loosening and mechanical stress have brought forth weaknesses. These faults come in gradually and get worse in time. Finding them out and repairing them requires continuous supervision and thus takes more time.

It also proved to require experience and tedious testing to build the manipulator grippers reliably and choose suitable sensors for the grippers.

Some of the auxiliary devices perform their tasks independently. Some of these operations are so critical that proceeding without feedback tends to cause problems in the measuring sequences if the independent operation fails.

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The moisture analyzer appeared to be the most demanding instrument to operate automatically as a part of this kind of a system. A pneumatic cylinder was built to open the cover of the analyzer. The dish with the sample on it is set to the scale by the manipulator, the cover is shut and when analyzing is finished the dish is again removed from the scale. It has proved to be difficult to handle the flat dishes with the grippers. Only very small tolerances are allowed in positioning the dish to and from the scale, otherwise either the scale is not in balance or the gripper does not recognize a dish. There was a malfunction situation where the gripper pulled the analyzer out of its place. After balancing the scale we took the instrument out of the robot system to avoid more crashes and re-designed the dishes and gripper sensors.

CONCLUSIONS

The main objectives of the project have been met despite the pioneer nature of the system. The analytical methods were validated and proved to be applicable. The efficiency and capacity have met with the expectations. Training of the operators and the maintenance people can be given by the plant's own personnel and the time needed for training is not essentially longer than in the other parts of the laboratory.

Simultaneously a LIMS system was built at the laboratory. Start-up of both of these systems has revealed some points, where more definitions and programming still has to be done. Such procedures are, for example, accepting or rejecting the final results, correcting wrong sample identifications and choosing the expression format for final results.

FURTHER DEVELOPMENT OF THE SYSTEM

The system was meant to release staff from routine work. This was necessary partly because some analysts were to retire. Now that the number of analysts in a shift is continuously reducing, services of the system become more valuable. Interruptions instantly cause delays, because the only alternative is manual analyses. More serious trouble in the system may cause a delay of hours due to repair work.

Despite the fact that many details still have to be improved, methods have been tested for analyzing sugar syrups and samples from the recovery stage of the refinery. The preliminary results have been promising.

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Table 1. Analytical methods of the robot system.

Analysis	Description of method
Dry substance	Density measurement Dry substance is calculated from density (by Handbook of Sugars)
Colour	The absorbance of a solution is measured at 420 and 720 nm, Sucros application of the "Bottlers method) (Cane Sugar Handbook)
Invert sugar	Enzymatic method, Boehringer-Mannheim enzymatic glucose-fructose-kit no. 139106, absorbance measurement at 340 nm
Moisture	Loss on drying, infra red (IR)- moisture analyzer
Ash	Conductivity ash, ICUMSA

Table 2. Description of analysing sequences. Dry substance value for calculation of colour, ash, invert is taken from the density measurement of the sample.

Analyses	Description of robot procedure
Dry substance	Density measurement Steps: Dosing - weighing - water dosing - weighing - mixing - measurement - washing
Colour	Liquids: Sample dosing - weighing - buffer solution dosing - mixing - measurement - washing
Invert sugar	Sugars: Lid open - water dosing - weighing - mixing - enzyme dosing - mixing - pause - measurement - washing
Moisture	Lid opening - emptying the cup to the dish - taking the dish to the analyzer - analysing - retrieving and emptying the dish
Ash	Sugars: Lid open - water dosing - weighing - mixing - conductivity measurement - washing

Table 3. Validation results of the methods.

Method	Reference method	Linear correlation factor r	Mean of differences paired-T-test
Moisture	Loss on drying, ICUMSA	0.76*	0.001%
Dry substance	Refractometric Dry Substance/ICUMSA	1	0.1%
Ash	Ash, manual conductivity, ICUMSA	1	0.000%
Invert Sugar	Invert Sugar/Lane-Eynon (ICUMSA)	0.91	0.03%
Colour	Colour/ICUMSA	0.98	1.5 IU

* Poor repeatability of reference method

Table 4. Fault classification during the first year of operation.
 *Only ten days of production in March.

Fault classification/ month	Month												Total
	6	7	8	9	10	11	12	1	2	3*	4	5	
Aux. devices and mechanics	24	35	29	6	18	3	3	7	2	-	6	9	142
Manipulator and grippers	11	12	6	5	9	6	-	1	2	-	1	4	57
Spectrofotometer	7	6	6	2	1	3	1	-	1	-	4	4	35
Density meter	14	15	13	8	3	4	1	7	4	-	4	4	77
Moisture analyzer	2	-	3	-	-	2	-	1	-	-	-	-	8
Conductivity meter	-	-	-	1	-	-	-	-	1	-	-	-	2
Electr.connect., press. air	3	-	1	1	-	-	3	1	-	-	-	-	9
Computer systems	4	-	7	1	4	-	-	1	1	-	-	4	22
User errors	3	2	3	-	2	-	-	-	-	-	-	-	10
Total per month	68	70	68	24	37	18	8	18	11	-	15	25	
Total per shift	1,1	1,0	1,0	0,4	0,6	0,3	0,2	0,4	0,2	0,0	0,3	0,4	

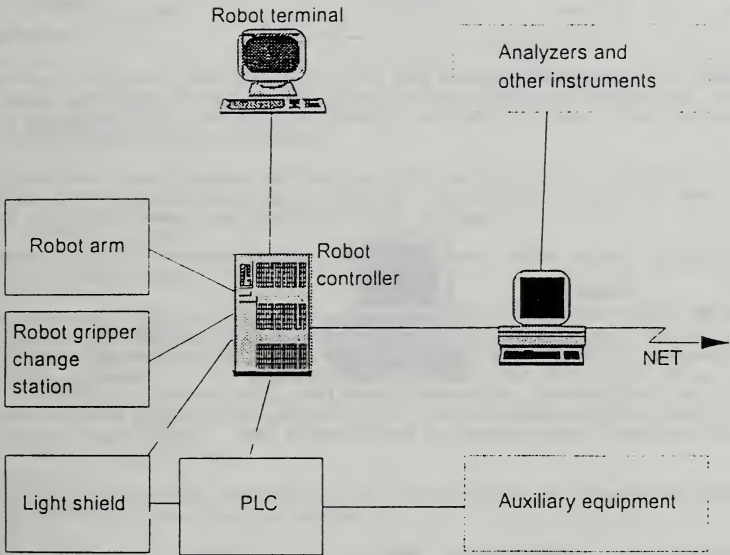


FIGURE 1: GENERAL SYSTEM LAYOUT

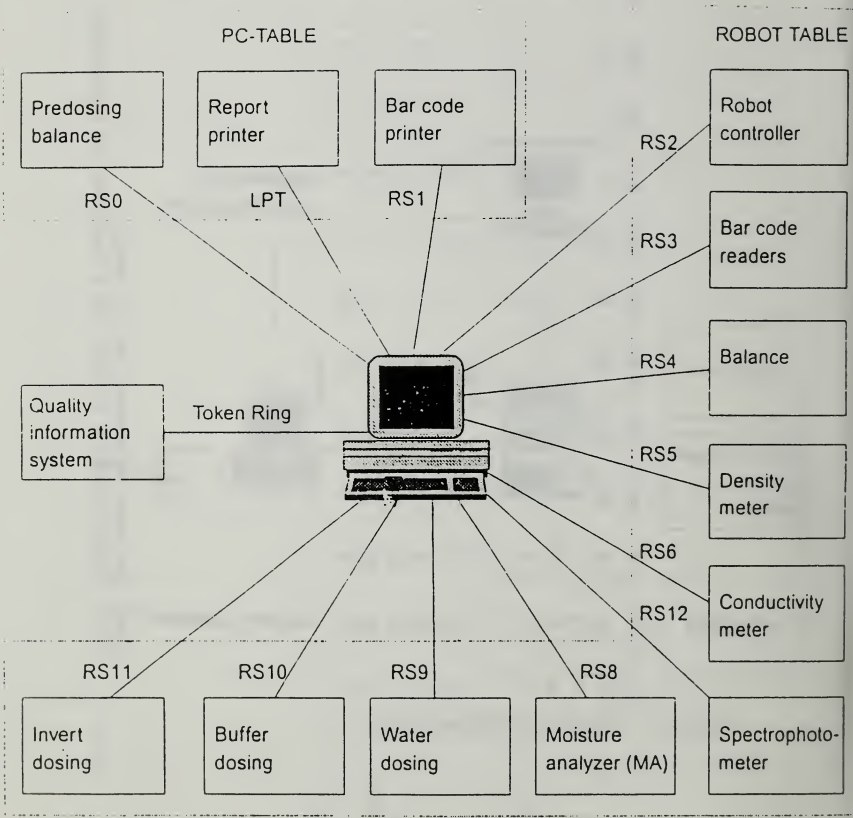


FIGURE 2: CONNECTIONS FOR THE ROBOT WORKSTATION PC

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Helsinki, Finland, August, 1994

FINNSUGAR SUCROS LTD. PORKKALA REFINERY

Tage Engman and Päivi Paakkari

Finnsugar Sucros Ltd., Helsinki, Finland

ABSTRACT

Porkkala Refinery was built in 1963 and operations started in 1965. Since raw sugar is mainly of Cuban origin, much emphasis has been put on decolorization by the design of the process. The process involves the following steps:

Affination: continuous affination and purging of massecuite on seven (7) discontinuous centrifugals. Affination syrup is sent to the recovery house, and boiled on the B-station.

Carbonatation: Carbonatation in two steps, with short retention time in both steps.

In first (I) filtration, mud is separated in six (6) Smith leaf-filters and sluiced with sweet water for desweetening outside the filter. Desweetening takes place in two (2) parallel automatic filter presses. In second filtration, the filtrate from I-filtration is treated with activated carbon for decolorization. The spent carbon powder and added filter aid is separated on four (4) GP-type bag filters. The filter cake is desweetened together with mud from I-filtration.

Decolorization: The decolorization takes place in an acrylic and a styrenic resin station in series.

Evaporation: The decolorized juice is evaporated to 73-74 Bx in a three step falling film evaporator before boiling.

Sugar House: White boiling in four (4) consecutive steps with no back or in boiling. Recovery station consists of three boilings A, B, and C. A-sugar is melted together with the affinated raw sugar. B- and C-sugars are melted together and returned to the A-boiling.

The capacity of the refinery is 650 t/d. The refinery is running five days per week. The yearly output varies from 100,000 to 120,000 tons.

Other products of the plant are: Liquid sugars from domestic beet sugar; Cube sugars, white and brown; Inverted syrups; Soft sugar;

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Brown dry crystal sugar; Nibs; Powder sugar; Food grade molasses for the industry.

Porkkala has a packaging department packing the products into a range of packages depending upon customer demands. There is also a printing shop on the site printing the main part of the packaging materials used in the sugar company.

The investment and development areas in recent years have been the following: Automation of the process; Working out and implementing a quality system based On ISO 9000 standards; Automation of laboratory routines by means of an automatic robot station.

The automation is realized throughout the whole process using a domestically made distributed automation control system. As a result the process is run by fewer personnel than before and the following up of the process is easy. The laboratory robot station will be covered in another paper.

In the paper more detailed figures and results will be presented and discussed.

INTRODUCTION

Porkkala refinery was originally designed to be able to handle raw sugar (RS) of lower quality. Much emphasis was put on decolorization. Colour removal takes place in a resin station and using activated carbon powder. Another feature of the refinery is the degree of integration between production lines with its benefits and its drawbacks.

In this paper the refinery process will be described along with some results and some analysis data. Data given correspond to refining cane raw sugar although domestic beet raw sugar also is used as raw material. When beet raw is used the process is simplified and some steps are bypassed. Figures represent the first half of 1994 where available. Some values derive from 1993. In appendices 1 to 3 there are diagrams for refinery process, liquid sugar production and syrup production respectively.

THE REFINERY PROCESS

Affination

Raw sugar and affination syrup are mixed and the magma is heated with warm water to 45-50°C before centrifugation. pH is adjusted to 6.5-7.0 by adding milk of lime to the mixer. Dry solids (DS) of magma is 91-92%. The magma is purged on six batch centrifugals,

600 kg magma per charge each, 1400 r/min. Affination syrup is sent to the B-boiling in the recovery house.

Melting

Affinated raw sugar is dissolved with sweet water to 68 Brix. The liquor passes through a mechanical strainer before it is pumped to the carbonatation.

Carbonatation

The carbonatation station consists of two tanks 18 m³ each giving a retention time of about 20 min each. The first tank is gassed to 9.2-9.4 pH and the second tank to 7.2-7.4 pH. Temperature is raised to 79-80°C by direct steam injection. Both tanks are equipped with an internal mixing pump.

Mud Separation

Mud is separated from the carbonatated liquor on six Smith pressure filters. The total filtering area is 402 m² (4330 sq.ft) representing 0.62 m²/TRS/d (6.7 sq.ft/TRS/d). The liquor feed is evenly distributed to the filters working. The total flow is regulated in conjunction to the liquor level in the feed tank. At the beginning of each filter cycle liquor is recirculated a certain time in order to minimize turbulence while precoating. When pressure drop over the filter reduces the flow to a preset minimum value, the cycle stops.

Mud is sluiced out of the filter with sweet water for desweetening. Polypropylene cloths are used in the Smith filters.

Mud Filtration

Mud is desweetened on two Larox automated filter presses. Total filter area is 92 m² or 0.14 m² TRS/D (219 sq.in/TRS/d). When used parallel, there is plenty of filter area available. The filter uses compressed air for the final drying of the cake.

Carbon Treatment

The filtrate from first filtration is mixed with activated carbon powder and diatomaceous earth and treated for about 40 minutes in a retention tank equipped with a stirrer. The reactor represents some compromise vis à vis plug flow and good mixing.

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Fine Filtration

The spent carbon is separated on four GP-type bag filters using the similar diatomaceous earth as precoat. Total filtering area is 200 m² (2150 sq.ft) which makes 0.31 m²/TRS/d (3.3 sq.ft/TRS/d.). The filter station is controlled in an analogue way to the first filtration. Here the emphasis is on a clear filtrate in order not to damage the following resin station.

Recirculation of filtrate takes place until this is clear whereafter the decision to proceed to the next step is made manually.

The mud from fine filtration is mixed with that from first carbonation for desweetening on the filter presses.

Resin Decolorization

The resin station consists of six flat-bottomed cells, containing 8 m³ resin each. The resin in three cells is of acrylic and three of styrenic type. Two acrylic cells are run in parallel as the first step and two styrenic ones as the second step. One acrylic and one styrenic unit are on stand-by or on regeneration. Regeneration takes place with sodium chloride solution containing 0.1 % sodium hydroxide. The liquid flow is downward as is the case in the regeneration.

Evaporation

The decolorized juice is evaporated from 64-65° Bx to 72-73° Bx in a three step falling film evaporator using turbine back pressure steam at 2 bar in the first step.

White Boiling

White boiling is carried out in four complete lines. Each line consists of a pan, massecuite mixer, centrifugal, sugar granulator and tank for spun off liquor. Each line can produce any grade of sugar. The equipment is interlocked to avoid mixing when changing from one grade to another in a certain line. A straightforward four boiling scheme is used. The four white grades are mixed together to produce a unit product. First grade is partly extracted for the production of high quality cube sugars. For the production of hard cube sugar a special double seeding procedure is used in order to get a large crystal distribution. Liquors are extracted (mainly from third boiling) for the production of syrups and brown sugars.

All pans are equipped with stirrers. Boiling is fully automated and consistency controlled using Berthold total density transmit-

ter. White massecuite is treated on five batch centrifugals, 600 kg each, 1200 r/min.

Recovery Station

Recovery is performed in three steps A, B, and C on two pans, 29 m³ each. Excessive syrup from affination is boiled without any further treatment together with the liquor from the A-strike in the B-step. A-massecuite is treated on a batch centrifugal and dissolved together with affinated raw. B- and C-massecuites are purged on continuous centrifugals and dissolved together and recrystallized in the A-strike.

LIQUID SUGAR PROCESS

Two different products are manufactured, liquid sucrose 65 and liquid 77, the latter being partly inverted.

For both products the raw material is domestic beet sugar of a good quality, low colour and ash. The raw material is selected during that period of the beet campaign when the floc level in sugar is low. In this way floc-free liquid sugar, that can be used also in the alcoholic beverage industry, is guaranteed. The process is on the other hand designed to take care of the specific beet sugar taste and odour.

The liquid 65 process is equipped with a granular carbon moving bed column and in the 77 process there is a granular carbon treatment. The inversion takes place on resin in acid form, with neutralization with caustic soda.

Materials and technical solutions by the installation have been chosen with care. Storage tanks are equipped with microfiltered air respiration.

SYRUP PROCESS

The syrup process has been renewed in 92 to 93 with the aim to raise the quality level of the products. The main raw material for syrup production is third white boiling run off liquor. Inversion is made with enzyme batchwise. There is no sterilization included in the process other than what the evaporation represents. After inversion different components are added depending upon which type of syrup is produced.

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RESULTS AND FIGURES

Some results and figures representing normal operational conditions during the first half of 1994 or 1993, by processing cane raw sugar, mainly of Cuban origin, will be presented.

Raw Material

Raw sugar (RS) varies in quality from one cargo to another. Whole raw colour varies from 3000 to 7000 ICU. Major problems occur, especially in filtration, when the raw sugar is of low microbiological quality. Precipitation with alcohol is also measured and it varies from 400 to 2700 ppm. A high value tends to indicate a lowered filterability.

In the results we will concentrate on colour, ash and to some extent invert. Average values for raw sugars used in 1994 are given in Table 1.

Affination

By purging the affination magma an average wash water amount of 0.8% on RS is used. This results in colour removal of 60% and ash removal of 57%. These figures include the colour and ash load which is recirculated to the melter via A-sugar and sweet water. Invert level is reduced by 64%.

Carbonatation

With a lime use of 7 kg /tRS colour removal in carbonatation is 63%, from 1798 ICU to 673 ICU.

Whenever the raw sugar is of such a quality that filterability of the liquor is bad, the use of lime tends to rise to 8-10 kg/tRS. The ash removal is about 20% and invert level falls by 27% in carbonatation.

Mud Desweetening

The washed and pressed mud, that is a mixture of carbonatation mud and fine filtration mud is disposed at a dry substance of 65%. Residual sugar content is 1% resulting a sugar loss of 0.013% on RS.

All sweet water resulting from mud desweetening is collected into the sweet water system.

Carbon Treatment

Activated carbon removes some colour but does also effect the taste and odour which is of importance when using beet raw sugar and especially when using sugar from molasses desugarization plant.

Colour removal is only 19%. Carbon use is 0.6 kg/TRS. Ash content before and after carbon treatment is not monitored. There is a small rise in invert content through the carbon treatment.

Resin Decolorization

In the resin station the main decolorization, 62%, takes place in the first cells which are loaded with acrylic resin. The second cells loaded with styrenic resin polish the liquor by removing a further 22% of the colour. Colour level of outcoming juice is held at less than 200 ICU. 164 has been the average. A normal feed used is 2.5 bed volumes/hour.

Colour loading expressed as IC-colour times bed volumes/cycle is by the acrylic cells fairly high, 126.000 ICBV/cycle and by the styrenic cells 48.300 ICBV/cycle. Cycle length is 230 BV/cycle giving a treatment length of 92 hours.

The regeneration is performed in series so that fresh brine is pumped through the styrene bed from where the used brine is fed to the acrylic bed. Salt consumption is 1940 kg/regeneration which makes 242 kg/m³ resin and 3.04 kg/TRS respectively.

There is some pick up of ash onto the resin. This is not measured but carbon treatment and resin station together lower the ash level by 7-8%. Invert content rises simultaneously by some 15%.

White Boiling

Prior to boiling the liquor is evaporated to 72-73° Bx. By evaporation the colour increases about 15-30 ICU which represent 10-20% increase in colour.

The colours of the four white grades in 1994 are given in Table 2.

Wash water to white centrifugals varies from 2-3.5% on sugar produced.

The fourth grade is partly used for soft brown sugar production. When the colour of the fourth grade sugar exceed 170-180 ICU it cannot be mixed with the first three grades to give a unit sugar having a colour of below 40 ICU. From time to time the fourth grade is thus dissolved and recrystallized.

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The colour increase over the whole white boiling varies from 100-200 % calculated from the 'colour mass balance' ($IC - \text{colour} \times \text{mass} \times Bx$).

Recovery House

Affination syrup is mixed into the spun off liquor from A-boiling. Purities and sugar contents through the recovery station are presented in Table 3.

Molasses is produced 2.5-3%/RS. Some liquors are extracted from the process for the production of syrups and brown sugar. This represents about 4%/RS.

The ratio invert to ash, in the fine liquor going to white boiling is 1.64, in the fourth spun off liquor going to recovery house 1.83, and in affination syrup 1.84. In the final molasses this ratio is 2.46 indicating that some inversion takes place throughout the process.

Other Results

Loss of dry substance throughout the refinery process taking into account the side streams into syrup production etc., calculated, from mass balance to be 0.77% on raw materials used.

The refinery steam use for all operations, including liquid sugar production and the production of special products and packaging, is 1.1 t/ton of product or 3.05 GJ/t product (847 kWh/t product). Consumption of electric power is 138 kWh per ton of product respectively.

Liquid Sugar

The raw material for liquid sugars is beet sugar of good quality. It can contain some undissolved material, which is filtered off in the liquid process. In Table 4 the analyses for raw material deriving from beet campaign 1993 are given.

In Table 5 the average analyses for liquid sugars produced during the first half of 1994 are explained.

The granular carbon for the liquid sucrose process is reactivated outside the process. Use of reactivated and fresh granular carbon is 2.5 kg/tDS.

INVESTMENTS

In the late eighties and the beginning of the nineties an automation investment program has been carried through. A Finnish made (Valmet) distributed automation and control system has been installed throughout the whole refinery. As a result the process is run by fewer persons than before. All in all the refinery employs 298 persons, when it runs in three shifts, five days a week. A new 4 MW turbine was installed in 1992/1993. The refinery can now produce about 80% of the total electric power consumption itself. At the time being a development program is running with the aim of working out and implementing a quality system based on ISO 9000 standards. The goal is to certify the production in 1995.

In the laboratory an automated robot station is installed. As a result the routine work of five chemists has been saved.

The syrup production has been modernized. Old and worn out equipment has been replaced by new ones and pipelines and tanks in stainless steel have been built. The process has not been changed but the end product is at a better microbiological level than earlier.

Technical data and production figures of the refinery are given in Appendix 4.

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Table 1. Average values of raw sugars used in 1994

Pol	97.9
pH	6.0
Ash cond., % of DS	0.35
Invert, % of DS	0.61
<u>Whole raw;</u>	
Colour, ICU	4429
Alcohol precipitate, ppm	1925
Undissolved material, g/kg	3.3

Table 2. Colours of white grade boilings, 1994

Grade	Colour Average	ICU Variation	CV
I	16	10-20	26.4
II	38	25-40	26.4
III	79	50-85	27.1
IV	199	140-220	28.2

Table 3. Purities and sugar contents in the recovery station

Syrup	Sucrose %/DS	Invert %/DS	ZN %/DS
Syrup to A-pan	90.5	3.3	93.8
Affination syrup	85.2	5.7	90.9
Syrup to B-pan	82.2	6.6	88.8
Syrup to C-pan	66.8	12.5	79.2
Refinery molasses	48.1	21.4	69.5

Table 4. Raw material for liquid sugar production from beet campaign 1993

Colour ICU	21	
Ash cond. %/DS	0.014	
Moisture %	0.016	
Alcoholic floc test	0.4	upper limit 2
Phosphoric acid floc test	0.9	upper limit 2

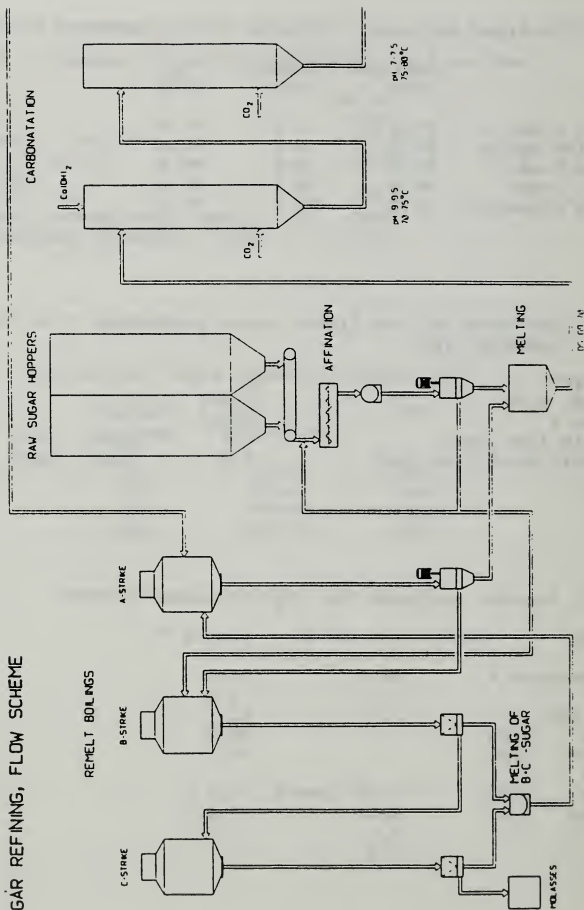
Table 5. Average analyses for liquid sugars in 1994

Analysis	Liquid sucrose 65	Liquid 77
Dry substance %	65.0	77.0
pH	5.9	4.2
Colour ICU	11	22
Sucrose %/DS		35.0
Fructose %/DS		31.1
+		
Glucose %/DS	0.23 invert	32.3
Ash cond. %/DS	0.03	0.03
Odour	0.1	0.01
Taste	0.1	-

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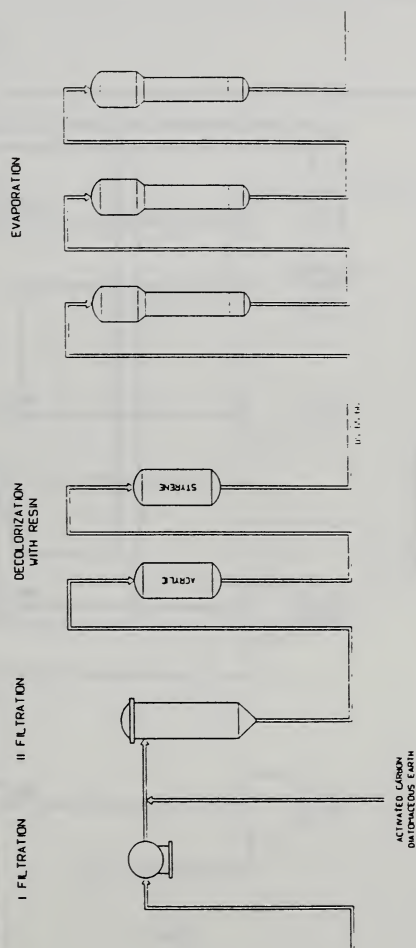
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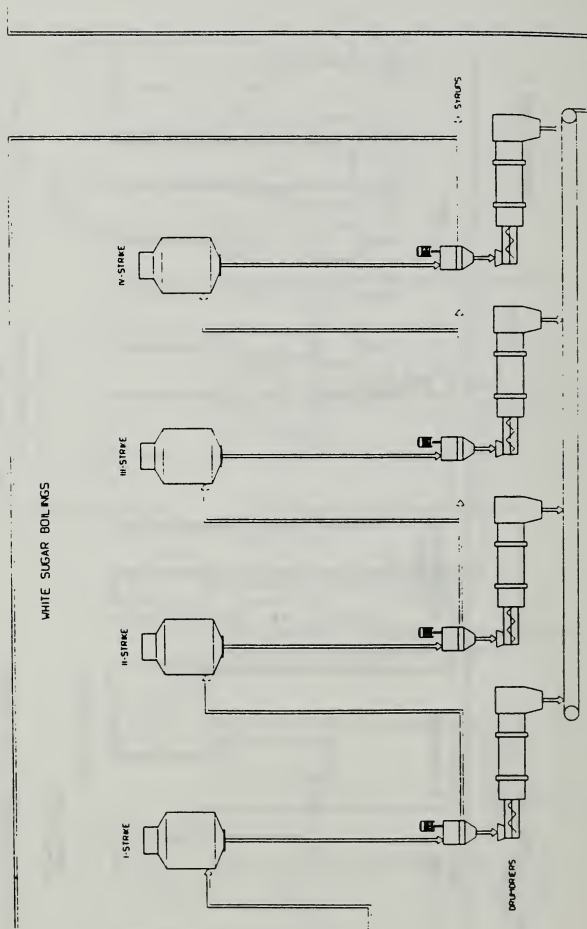
SUGAR REFINING, FLOW SCHEME



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Appendix 1 page 2

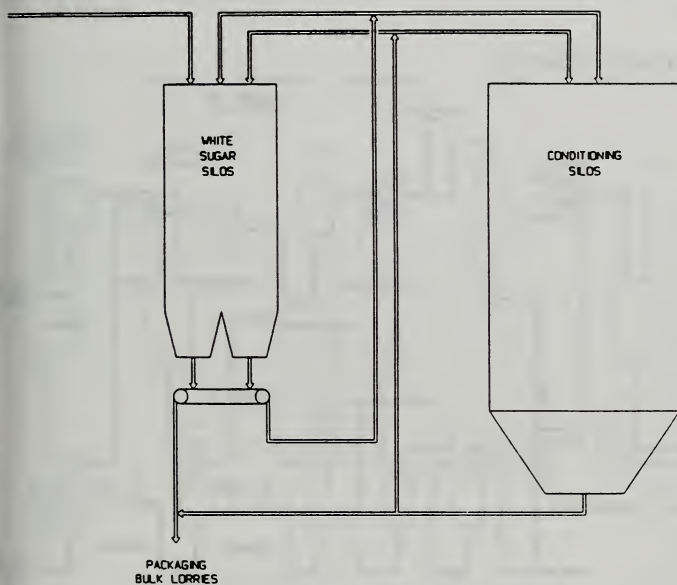




1994

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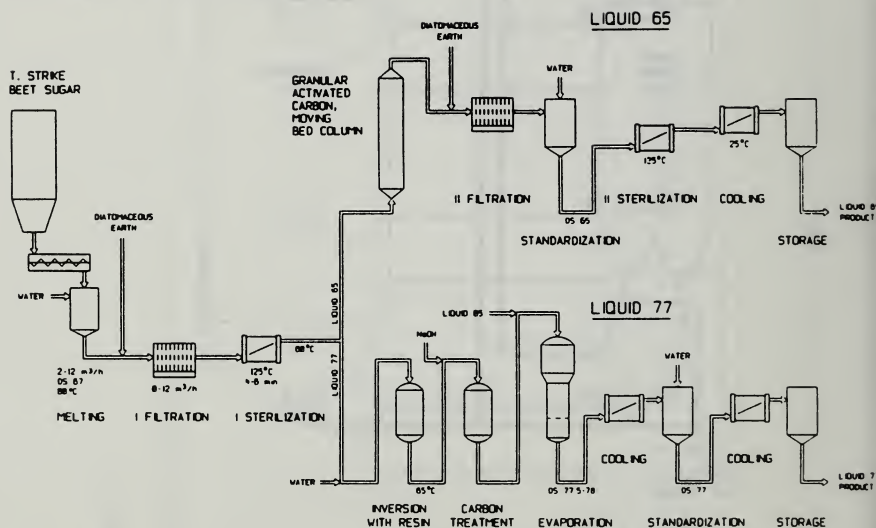
Appendix 1 page 4



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POROKALA REFINERY

Appendix 2

FLOW SCHEME OF LIQUID SUGAR PRODUCTION



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Appendix 3

FLOW SCHEME OF SYRUP PRODUCTION

BLENDING

FILTRATION

EVAPORATION

COOLING

STANDARDIZATION

STORAGE

3 RD STRIKE
RUN OFF FROM
REFINERY

OTHER
COMPONENTS

WATER

INVERSION
WITH ENZYME
pH 5.0 - 5.5
T 60°C
DS 95

DS 60

3.4 1/h

40°C

DS 90

SYRUP
PRODUCTIONS

Technical data

Raw material	
The refinery used raw material in 1993 as follows:	
	1000 tons
* Domestic beet white sugar directly to packaging	22
* Industrial grade beet white sugar for liquid sugar production	35
* Beet raw sugar for refining	28
* Sugar from molasses desugarization plant in Naantali	6
* Imported raw sugar: cane	98
beet	4
TOTAL	193

Capacities	t/d	Storage facilities	
Refining	650	Raw sugar silos	100.000 t
Liquid sugars	200	White sugar silos	
Syrups	50	3x3500 t	10.500 t
Products	1000 t/a	Storage tanks	29.000 m ³
Crystalline sugars	110-140		
Liquid sugars	35	Other activities	
- Liquid 65		Package printing plant	
- Liquid 77		Fresh water purification plant,	
Cube sugars	15	capacity max 7200 m ³ /d	
Special products	9,5	Waste water purification plant,	
- Jam sugar		capacity max 2100 m ³ /d	
- Jelling sugar		Power plant:	
- Brown soft sugar		- Steam power max 50 MW (30 Bar)	
- Brown dry sugar		- Electric power 4 MW	
- Icing sugars			
- Nibs			
Syrups	7,0		
Export	15-45		

DISCUSSION

Question: Can we have copies of your transparencies, on consumption, etc.?

Engman: Yes, of course.

Question: Can you tell us why you prefer to use beet sugar to make liquid sugar?

Engman: The main reason is to enable production of floc-free liquid sugar for all our customers. We sell sugar to the alcoholic beverage producers, and these customers react easily to alcoholic floc. We could also make liquid sugar from cane, but we could not guarantee that to be floc free, every day, so the use of beet sugar is a practical way to overcome this problem.

Comment: Another reason why we use beet sugar is that it enables us to store the beet sugar (almost white) which is produced in our beet sugar factories in simple storage (raw sugar storage type). Otherwise we would have to invest in silos.

ABSTRACTS OF POSTER PRESENTATIONS

THE MEASUREMENT OF DEXTRAN IN RAW SUGARS USING ¹H NMR
Les A. Edye¹, Shaoxiong Wu², and Margaret A. Clarke¹.

¹Sugar Processing Research Institute, Inc., New Orleans,
Louisiana, USA

²Tulane University, New Orleans, Louisiana, USA

In the cane sugar industry the purchase price of raw cane sugar, the product of sugarcane processing, is determined by polarimetric measurement of sucrose (Pol). Raw sugar is greater than 96% sucrose, but it also contains other saccharides and non-sugars which can contribute to Pol. Dextran, one class of polysaccharides often found in raw sugar, effect an increase in pol and interfere with subsequent refining. The U.S. sugar refining industry can impose a penalty on the raw sugar purchase price for high dextran content. While there are several wet chemical methods for the determination of dextran in raw sugar the results of these analyses are rarely in agreement.

This presentation reviews existing wet chemical methods for dextran determination and compares the results of these analyses with the results obtained from the physical measurement of dextran in raw sugar by ¹H NMR spectroscopy.

NEAR INFRARED ANALYSIS OF SIMPLE AND COMPLEX SACCHARIDES

Margaret A. Clarke¹, Les A. Edye¹ and Cynthia McDonald-Lewis²

¹Sugar Processing Research Institute, Inc., New Orleans,
Louisiana, USA

²NIRSystems, Inc., Silver Spring, Maryland, USA

Near infrared (NIR) scanning spectroscopy has been applied to analysis of sugarbeet and sugarcane juices, syrups, sugars and byproducts for determination of simple sugars (sucrose, glucose, fructose) and polysaccharides (starch, dextran).

Development of analytical methods in the laboratory, field and factory is described. Input data for preparation of robust calibrations were acquired in multiple locations over several crop years, so that varying crop conditions were taken into account. Calibrations were verified similarly, in a variety of geographic areas. Hazards that may occur in development of these calibrations are

noted. Examples of application of these calibrations to analysis in sugarcane and sugarbeet factories and refineries are presented. On-line applications are outlined. The ability to run simultaneous determinations of sugars by several different methods through the use of NIR analysis is discussed.

ISOLATION AND CHARACTERIZATION OF DEXTRANS FROM SUGARBEET AND BEET SUGAR

Margaret A. Clarke¹, Per J. Garegg² and Earl J. Roberts¹.

¹Sugar Processing Research Institute, Inc., New Orleans, Louisiana, USA

²Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden

Sugarbeets harvested under conditions of high ambient temperature, or after freezing and thawing, have been observed to yield juices of high viscosity that are difficult to filter in factory processing. These processing problems have been attributed to microbial polysaccharides, usually of the levan type, formed on the damaged sugarbeet and then dissolved in the juice.

Sugars and juices from heat or freeze-damaged sugarbeets were obtained. Polysaccharides were isolated from these samples by alcohol precipitation and dialysis procedures. Analysis by ¹³C and ¹H nmr identified the isolated polysaccharides as dextrans. These dextrans showed a high degree of linearity, with $\alpha(1\rightarrow6)$ backbone, and $\alpha(1\rightarrow4)$ branch points in amounts varying from 1% to 5%. In structure, the dextrans appear similar to dextran produced by Leuconostoc mesenteroides B512-F.

HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY) ANALYSES OF SUGARBEET JUICES

Rebeca S. Blanco, Chad V. Scott, Les A. Edye and Margaret A. Clarke

Sugar Processing Research Institute, Inc. New Orleans, Louisiana, USA

Analysis of beet juices by cation exchange HPLC sometimes gives results that show discrepancies from traditional pol, Brix and purity measurements. Reasons for the discrepancies are outlined. The use of alternate analytical systems that can be used to determine sources of discrepancy and error are demonstrated.

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A NEW INFRARED DRIER FOR WHITE, RAW, AND POWDERED SUGARS

Xavier M. Miranda, Mary An Godshall, and Margaret A. Clarke

Sugar Processing Research Institute, Inc. New Orleans, Louisiana, USA

An infrared drier for solid sugar products was, in trials at SPRI, tested on raw, white, and powdered sugars. Results are reported and compared to standard oven drying procedures. Modifications to suggested methodology are discussed.

The system requires less than 10 minutes drying time, compared to the traditional 3-4 hours.

BIOSENSOR ELECTRODE (BSE) FOR DETERMINATION OF SUCROSE AND GLUCOSE

Rebeca S. Blanco, Les A. Edye and Margaret A. Clarke

Sugar Processing Research Institute, Inc. New Orleans, Louisiana, USA

A new enzyme-based electrode system for determination of sucrose and glucose is available (Orion Research Co.). The electrode, which holds enzyme in a carbon paste medium, was tested on factory and refinery samples at SPRI. Results on ranges of application and comparison to other analyses for sucrose and glucose are presented.

ANALYSIS OF OLIGOSACCHARIDES IN SUGAR PRODUCTS AND HONEY BY HIGH PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION (HPAEC-PAD)

Margaret A. Clarke, Les A. Edye and Glori-Lynn Cargel

Sugar Processing Research Institute, Inc., New Orleans, Louisiana, USA

Separation of 1-, 6-, and neo-kestose and raffinose by HPAEC-PAD (also called ion chromatography) is described. These oligosaccharides are separated from sucrose, glucose and fructose in products of sugarcane and sugarbeet (juices, sugars, molasses).

Separation of oligosaccharides in honey by HPAEC-PAD is described. After isolation by carbon column treatment, oligosaccharides from honey can be analyzed, for purposes of comparison with beet sugar oligosaccharides, in less than 30 minutes.

A CONTRIBUTION FROM SUCROSE TO CHEMICAL THEORY*

Alfred D. French, Southern Regional Research Center, U.S. Department of Agriculture, P.O. Box 19687, New Orleans, Louisiana 70179, USA

Sucrose is unlike many other common disaccharides because it is a non-reducing sugar, with its two anomeric carbon atoms linked to a shared glycosidic oxygen atom. Also, unlike results of studies on many other disaccharides, molecular modelling has been unable to account for the observed relative orientations of the fructose ring about its bond to the glycosidic oxygen atom. Those studies were based on molecular mechanics modelling, a method that is based on existing knowledge of bond lengths, bond angles, torsional potentials, etc. A new study using quantum mechanics was able to account for the observed shapes of the sucrose moiety. This shows that additional chemical theory must be developed to properly depict the torsional behavior for molecules such as sucrose that have adjacent anomeric centers.

* Adapted from "Ab Initio-MIA and Molecular Mechanics Studies of the Distorted Sucrose Linkage in Raffinose", Christian Van Alsenoy, Alfred D. French, Ming Cao, Susan Q. Newton, and Lothar Schäfer, *Journal of the American Chemical Society* 1994, 116, 9590-9595.

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CONFERENCE ON SUGAR PROCESSING RESEARCH
KALASTAJATORPPA HOTEL
HELSINKI, FINLAND

August 7-9, 1994

ACTUAL ATTENDANCE LIST

- Andersson, Visti, Sales Director, NIRO-DDS, 305 Gladsaxevej,
DK-2860 Soeborg, Denmark
- Awad, Ahmed, Vice President, Research and Development, Domino
Sugar Corp., 266 Kent Ave., Brooklyn, NY, USA 11211
- Balogh, Theresa, Chief Chemist, Redpath Sugars, 95 Queens Quay
E., Toronto, M5E 1A3, Canada
- Bekker, P. I., Manager, Development, S.A.S.A./SMRI, Transvaal
Sugar Ltd., Malelane, South Africa 1320
- Bennett, Michael C., (former President of Sugar Processing
Research Institute, Inc.), Clippings Green Farm, Dereham,
Norfolk NR20 3RG, UK
- Bento, Luis R.S.M., Technical Director, RAR - Refinarias de Acucar
Reunidas, SA, Apartado 1067, Porto 4104, Portugal
- Berghäll, Sune, Sucros Ltd., Salo Factory, Sokerikatu 1,
FIN-24100 Salo, Finland
- Blanco, Rebeca S., Laboratory Technician, Sugar Processing Research
Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, LA,
USA 70124
- Blok, Jacob, Director, Technics and Technology, CSM Suiker bv,
P. O. Box 3224, 4800 DeBreda, The Netherlands
- Bly, Mirja, Xyrofin Ltd., Sokeritehtaantie, FIN-48210 Kotka,
Finland
- Broughton, N. W., Director, Research and Development, British Sugar
plc, Technical Centre, Norwich Research Park, Norwich NR4 7UP,
UK
- Brown, C. R. (Dick), Vice President, Quality Assurance, Lantic
Sugar, 4026 Notre Dame St. East, Montreal, Quebec H1W 2K3,
Canada
- Bruce, Pia, Cultor, Ltd., Technology Center, FIN-02460 Kantvik,
Finland

- Bruhns, Jürgen, Editor, Zuckerindustrie, Lückhoffstr. 16, 14129 Berlin, Germany
- Burge, Malcolm L., Central Q. A. Manager, Tate & Lyle Sugars, Thames Refinery, Factory Rd., London E16 2EW, England, UK
- Chou, Chung-Chi, Technical Director, Domino Sugar Corporation, 1114 Avenue of the Americas, New York, NY, USA 10036-7783
- Clarke, Margaret A., Managing Director, Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, LA, USA 70124
- Connolly, Anthony A., Director of Operations, Irish Sugar plc, Athy Rd., Carlow, Ireland
- Costesso, Dennis, Vice President, Research & Quality Assurance, The Amalgamated Sugar Co., 2427 Lincoln Avenue, Ogden, UT, USA 84401
- Davies, V. R. (Bill), Sugar Market Specialist, Rohm & Haas Co., Hilltop Executive Centre, #207, 1590 S. Milwaukee Ave., Libertyville, IL, USA 60048
- de Bruijn, Jan Maarten, CSM Suiker bv, Central Laboratorium, P. O. Box 3248, Valveken 6, 4800 DeBreda, The Netherlands
- Desai, Snehal, Market Development Manager, Dow Chemical Company, Larkin Laboratory, Midland, MI, USA 48674
- Donnelly, C. R. (Dick), Sr. Vice President of Operations, Savannah Foods and Industries, P. O. Box 710, Savannah, GA, USA 31498
- Donovan, Mike, R&D Manager, Tate & Lyle Sugars, Plaistow Wharf, North Woolwich Road, London E16 2AG, England, UK
- Edye, Les A., Senior Research Scientist, Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, LA, USA 70124
- Engman, Tage, Sucros Ltd., Porkkala Refinery, FIN-02460 Kantvik, Finland
- Erjala, Matti, Researcher, Finnsugar Ltd., Sugar Beet Research Centre, Korvenkyläntie 201, FIN-25170 Kotalato, Finland
- Farstrup, Karin, Technical Product Manager, Danisco A/S, Langebrogade 1, DK-1001 Copenhagen, Denmark

SPRI

Foster, Bruce, Executive Director, Sugar Industry Technologists,
P. O. Box B-2, RR#2, Lost River, Quebec J0S1 1A0, Canada

French, Alfred D., Research Chemist, Southern Regional Research
Center, ARS-USDA, P. O. Box 19687, New Orleans, LA, USA
70179

Galt, Sarah, Project Leader, Dow Chemical, Larkin Laboratory,
Midland, MI, USA 48674

Gannon, Sean, Technical Director, Irish Sugar plc, Athy Road,
Carlow, Ireland

Garegg, Per J., Professor, Dept. of Organic Chemistry, Arrhenius
Laboratory, S-106 91, Stockholm, Sweden

Godshall, Mary Ann, Sr. Research Associate, Sugar Processing
Research Institute, Inc., 1100 Robert E. Lee Blvd., New
Orleans, LA, USA 70124

Gudmundson, Claes, Head of Department, Development Center,
Sockerbolaget, Box 202, Svedala 233 24, Sweden

Guglielmi, Phil, Quality and Environmental Manager, Redpath Sugars,
95 Queens Quay E., Toronto, Ontario M5E 1A3, Canada

Guimarães, Carla, RAR - Refinarias de Acucar Reunidas, SA, Apartado
1067, Porto 4101, Portugal

Hallanoro, Helena, Cultor Ltd., Finnsugar Bioproducts, Satamatie
2, FIN-21100, Finland

Hinkkanen, Ritta, Research Manager, Cultor Ltd., Technology Center,
FIN-02460 Kantvik, Finland

Huttunen, Hannu, Plant Manager, Sucros Ltd., Sakyla Plant, P.O. Box
210, FIN-27820 Iso-Vimma, Finland

Hyöky, Göran, Finnsugar Ltd., Finnsugar Development, FIN-02460
Kantvik, Finland

James, Alan (Past President CSRRP), 16, Crossbush Road, Felpham,
Sussex PO22 7L5, UK

Jansdorf, Henrik, Executive Vice President, De Danske
Sukkerfabrikker, Langebrogade 1, P.B. 17, Copenhagen K,
Denmark 1001

- Jensen, John, Section Manager, Danisco A/S, De Danske Sukkerfabrikker, DDS Development Center, Maribovej 2, Nakskov, DK-4900, Denmark
- Johnson, Lance, Dow Chemical, Industrie Strasse 1, Rheinmuenster D-77836, Germany
- Jonasson, Richard, Quality Coordinator, Sockerbolaget AB, Arlöv Sugar Refinery, P. O. Box 32, Arlöv S-23221, Sweden
- Jones, Russell, Analytical Development Scientist, British Sugar plc, Norwich Research Park, Colney, Norwich NR4 7UB, UK
- Kaipainen, Antti, Research Chemist, Cultor Ltd., Technology Center, SF-02460 Kantvik, Finland
- Kartasasmita, Soedjai, Honorary Chairman, IKAGI (Indonesian Society of Sugarcane Technologists, Jl. Iskandarsyah II/62 Kebayoran Baru, Jakarta-Indonesia 12160
- Kernchen, Wolfgang, President, Dr. Wolfgang Kernchen GMBH, P. O. Box 20140, D-30921 Seelze, Germany
- Khan, Riaz, POLYbios, Padriciano, 99 1, 34012 Trieste, Italy
- Koay, Moi Yim, Chemist, Malayan Sugar Manufacturing Co., 798 Main Road, Prai, Penang 13600, Malaysia
- Kohijoki, Irmeli, Sucros Ltd., Salo Factory, Sokerikatu 1, FIN-24100 Salo, Finland
- Kunze, Nicholas, Senior Applications Engineer, Chemvicon Carbon, Boulevard de la Woluwe, Nr. 60 - BTE 7, Brussels, Belgium
- Kuusisto, Juhani O., President, Finnsugar Sucros Ltd., Sokerikatu 1, 24100 Salo, Finland
- Lee, Edward, Senior Physical Chemist, Tate & Lyle Sugars, Plainstow Wharf, North Woolwich Road, London, England E16 2AG, UK
- Lichtenthaler, Frieder, Professor, Institute für Organische Chemie, Darmstadt Technical University, Darmstadt, Petersenstr. 22, Germany
- Liljeqvist, Selja, Quality Assurance Manager, Xyrofin Ltd., Sokeritehtaanatie, FIN-48210 Kotka, Finland
- Lundahl, Karl, Development Engineer, Sockerbolaget AB, Arlöv Sugar Refinery, Box 32, Arlöv S-232 21, Sweden

SPRI

Mantovani, Giorgio, Professor, University of Ferrara, Via L. Bosari
46, 44100 Ferrara, Italy

Marjamaa, Martti, Research Chemist, Cultor Ltd., Technology Center,
FIN-02460 Kantvik, Finland

Mathlouthi, Mohamed, Professor, University of Reims, Moulin de la
Housse, B.P. 347, F-51062 Reims Cedex, France

Mattila, Lea, Process Manager, Xyrofin Ltd., Sokeritehtaan-
tie, FIN-48210 Kotka, Finland

McDonald-Lewis, Cynthia, Scientist, Tecator AB/NIRSystems,
12101 Tech Road, Silver Spring, MD, USA 20904

McReynolds, Kent, Development Associate, Dow Chemical, Larkin
Laboratory, Midland, MI, USA 48674

Mont, Rick, Process Research Manager, American Crystal Sugar
Company, P. O. Box 1227, Moorhead, MN, USA 56561-1227

Mota, Manuel, RAR - Refinarias de Acucar Reunidas, SA,
R. Manuel Pinto de Azevedo, 272, Porto 4100, Portugal

Nouvel, Francois, Responsable-Direction Technique, Generale
Sucriere, 106 Rue du Maréchal Leclerc, Eppeville 80400, France

Nurmi, Hans, Sucros Ltd., Turenki Plant, Harvialantie 13, FIN-14200
Turenki, Finland

Nurmi, Juha, Finnsugar Ltd., Finnsugar Development, FIN-02460
Kantvik, Finland

Nygren, Eino, Sucros Ltd., Salo-Turenki Unit, Salo Factory,
Sokerikatu 1, FIN-24100 Salo, Finland

Olofsson, Birgitta, Chemical Engineer, Process Development
Refinery, Sockerbolaget AB, Arlöv Sugar Refinery, Box 32,
Arlöv 23221, Sweden

Ong, Yong Fatt, Process Manager, Malayan Sugar Manufacturing
Co. GHO, 798 Main Road, Prai, Peking 13600, Malaysia

Paakkari, Päivi, Sucros Ltd., Porkkala Refinery, FIN-02460
Kantvik, Finland

Paldanius, Jorma, Sucros Ltd., Salo Plant, FIN-24100 Salo, Finland

Paananen, Hannu, Manager, Process Technology, Cultor Ltd.,
 Finnsugar Bioproducts, Inc., Kyllikinportti 2, FIN-00241,
 Helsinki, Finland

Parkin, Geoff, Laboratory Manager, British Sugar plc, Norwich
 Research Park, Colney, Norwich NR4 7UB, UK

Patout, William S., III, President & C.E.O., M. A. Patout &
 Son, Ltd., 3512 J. Patout Burns Rd., Jeanerette, LA, USA 70544

Pelo, Marja, Laboratory Manager, Finnsugar Ltd., Sugar Beet
 Research Centre, Korvenkyläntie 201, FIN-25170 Kotalato,
 Finland

Pelto, Armi, Sucros Ltd., Turenki Plant, Harvialantie 13, FIN-14200
 Turenki, Finland

Persson, Raland, R&D Manager, Sucros Ltd., Sakyla Plant, P.O. Box
 210, FIN-27820 Iso-Vimma, Finland

Purchase, Brian S., Sugar Milling Research Institute, University of
 Natal, Durban, South Africa 4001

Puuppo, Outi, Finnsugar Ltd., Finnsugar Development, FIN-02460
 Kantvik, Finland

Raininko, Kyösti, Director, Finnsugar Ltd., Sugar Beet Research
 Centre, Korvenkyläntie 201, FIN-25170 Kotalato, Finland

Rajakaylä, Eero, Xyrofin Ltd., Sokeritehtaantie, FIN-48210 Kotka,
 Finland

Ramm-Schmidt, Leif, President, Hadwaco Ltd Oy, Emalikatku 10,
 FIN-004400 Jarvenpaa, Finland

Rearick, Gene, Group Leader, Amalgamated Sugar Co., P. O. Box 127,
 Twin Falls, ID, USA 83303

Reiser, Philippe, Engineer, CEDUS, 30 Rue de Lübeck, F-75116 Paris,
 France

Rinkinen, Raili, Cultor Ltd., Kyllikinportti 2, FIN-00240 Helsinki,
 Finland

Roberts, Earl J., Senior Research Chemist, Sugar Processing
 Research Institute, Inc., 1100 Robert E. Lee Blvd., New
 Orleans, LA, USA 70124

SPRI

Rosengvist, Robert, Plant Manager, Cultor Ltd., Finnsugar Bioproducts, Inc., Kyllikinportti 2, FIN-00241 Helsinki, Finland

Sarkki, Marja-Leena, Finnsugar Ltd., Finnsugar Development, FIN-02460 Kantvik, Finland

Saska, Michael, Associate Professor, LSU Agricultural Center, Sugar Station Bldg., LSU, Baton Rouge, LA, USA 70803

Schwartz, Thomas K., Executive Vice-President, Beet Sugar Development Foundation, 90 Madison St., Denver, CO, USA 80206

Scott, Chad, Technician, Sugar Processing Research Institute, 1100 Robert E. Lee Blvd., New Orleans, LA, USA 70124

Sinclair, Keith, Technical Services Manager, Tate & Lyle Sugars, Factory Road, Silvertown, London, England E16 2EW, UK

Somaruga, Giovanni, Dow Italia SPA, Via G. Murat, 23, Milano 20159, Italy

Steinle, Georg, Abteilungsleiter, Sudzucker AG Mannheim/Ochsenfurt, Obbrigheim 67283, Germany

Strickland, Robert W., Vice President Operations, Holly Sugar Corporation, P. O. Box 1052, Colorado Springs, CO, USA 80901

Svård, Christer, Project Manager, Sockerbolaget AB, Box 32, S-23221 Arlöv, Sweden

Talvitie, Erkki, Sucros Ltd., Porkkala Plant, FIN-02460, Kantvik, Finland

Theobald, Trevor C., Senior Research Scientist, British Sugar Research & Development, Technical Center, Norwich Research Park, Norwich NR4 7UB, UK

Tilmanis, Leif, Product Specialist, Tecator AB, Box 70, Höganäs S-26321, Sweden

Triche, Mark P., Comptroller, Savannah Foods & Industries, P. O. Box 710, Savannah, GA, USA 31402

Tylli, Matti, Finnsugar Ltd., Finnsugar Development, FIN-02460 Kantvik, Finland

Vaccari, Giuseppe, Professor, University of Ferrara, Via L. Borsari, 46, Ferrara, Italy 44100

1994

Vernon, Robin, Managing Editor, International Sugar Journal, P. O. Box 26, Port Talbot, West Glamorgan, UK

Viljanmaa, Jussi, Sucros Ltd., Porkkala Refinery, FIN-02460 Kantvik, Finland

Viljava, Tapio, Cultor Ltd., Technology Center, FIN-02460 Kantvik, Finland

von Weissenberg,, Robert, Cultor Ltd., Technology Center, FIN-02460 Kantvik, Finland

Walliander, Pertti, Project Manager, Cultor Ltd., Technology Center, FIN-02460 Kantvik, Finland

Wen, Tong, Cultor Ltd., Technology Center, FIN-02460 Kantvik, Finland

Wendel, Lars, Manager, Quality Control, Sockerbolaget AB, Arlöv Sugar Factory, P. O. Box 32, Arlöv S-23221, Sweden

Williams, John, Consultant, Tate & Lyle Sugars, 5 Pigott Road, Wokingham, Berks RG11 1P2, UK

Ylisuutari, Soili, Sucros Ltd., Porkkala Refinery, FIN-02460 Kantvik, Finland

1. The first part of the paper discusses the importance of the study and the objectives of the research. It also mentions the scope of the study and the limitations. The second part of the paper discusses the methodology used in the study. It includes the data collection methods, the sample size, and the statistical methods used for data analysis. The third part of the paper discusses the results of the study. It includes the findings of the study and the conclusions drawn from the results. The fourth part of the paper discusses the implications of the study and the recommendations for future research. The fifth part of the paper discusses the limitations of the study and the suggestions for future research. The sixth part of the paper discusses the conclusions of the study and the recommendations for future research. The seventh part of the paper discusses the limitations of the study and the suggestions for future research. The eighth part of the paper discusses the conclusions of the study and the recommendations for future research. The ninth part of the paper discusses the limitations of the study and the suggestions for future research. The tenth part of the paper discusses the conclusions of the study and the recommendations for future research.

